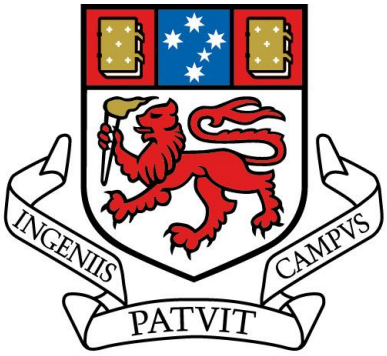

UNIVERSITY OF TASMANIA

Miniaturised Liquid Chromatography

By

Yan Li

A dissertation submitted in fulfilment of the requirements for the Doctor of Philosophy

(Chemical Sciences)

School of Natural Sciences

University of Tasmania

Oct 2018

Declaration of Originality

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Acknowledgement

This thesis could not be realised without the support and help from many individuals. I would like to express my sincerest appreciation and respect to my primary supervisor, Professor Mirek Macka, for his guidance and mentorship during this PhD. Thank you for believing in my abilities, providing me with enormous flexibility and independence in my research, and encouraging me to express and pursue my own ideas. Mirek was not just my supervisor, but also a friend. I enjoyed numerous after-work discussions of various topics and was always impressed by his wisdom and knowledge. Sincere thanks to my secondary supervisors Professor Pavel Nesterenko, Professor Brett Paull and Professor Roger Stanley for expert advice, untiring support and guidance throughout this project. Professor Pavel Nesterenko and Professor Brett Paull also supervised my earlier Master of Science work. Under their supervision, I have developed good research practices that will follow me through the rest of my life.

Thank you to all staff within the Discipline of Chemistry at the University of Tasmania and the Australian Centre for Research on Separation Science for their academic and technical support. In particular to Dr Miloš Dvořák, Dr Nantana Nuchtavorn, Dr Min Zhang, Dr Anton Peristyy, Dr Estrella Sanz Rodriguez, Dr Mohammad Talebi and Dr Petr Smejkal, for their kind help with the operation of analytical equipment. My special thanks to Murray Frith, (Laboratory Manager), for assuring a smooth and comfortable working environment. With Murray's support, I undertook the health and safety representative role at the Discipline of Chemistry, giving me the opportunity to engage in developing and projecting health and safety policies. I would like to acknowledge my host Dr Hong Heng See from the University of Technology Malaysia, for the

opportunity to visit his laboratory for 3 weeks, which to extend my skills in 3D printing technologies. I would also acknowledge the cooperation and help of LabSmith with instrumental and software support, namely, Kirsten Pace.

I would also like to acknowledge the people who were always there for me. Professor Michael Breadmore guided me when I struggled to see the direction in my PhD, treated me as a member of his research group and looked out for me during numerous international conferences. I also would like to thank Father Michael Tate and Father Brian Nichols from Sandy Bay Parish, Tasmania, they both provided countless help during my life in Tasmania. The friendships with Mrs Brenda Mooney, Dr Min Zhang, Dr Hong Heng See, Dr Feng Li, Dr Yabin Wen and Mr Shing Chung Lam, grew from fun times in and after work, and will last forever.

I want to say XieXie (thank you) to my parents, Zhongli Li and Wen Yan, for having me, standing with me in all moments of life and supporting all my endeavours. Though my parents desperately wish me to stay in China with them, I am grateful for unconditionally supporting my decision to pursue life in Australia. Last but not least, I would like to thank my wife Mónica Alves for her understanding, patience and love.

Lots of good old days nostalgic memories came out while writing this acknowledgement. I deeply cherish this unforgettable and important four years in my life, which I had many great experiences and grew up a lot. Now is the end but also is a beginning, “so we beat on, boats against the current, borne back ceaselessly into the past” (Francis Scott Key Fitzgerald).

Statement of Co-authorship

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

In Chapter 2, Paper 1: Li, Y.; Pace, K.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Miniaturised electrically actuated high pressure injection valve for portable capillary liquid chromatography. *Talanta* **2018,** *180*, 32-35.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Kirsten Pace, Mirek Macka, Pavel Nesterenko, Brett Paull and Roger Stanley contributed to the idea, its formalisation and development. All co-authors assisted with refinement and presentation.

In Chapter 3, Paper 2: Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Comparison of cation-exchange capillary columns used for ion chromatographic separation of biogenic amines. *Journal of Chromatography A* **2018,** *1571*, 193-200.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Mirek Macka, Pavel Nesterenko, Brett Paull and Roger Stanley contributed to the idea, its formalisation and development. All co-authors assisted with refinement and presentation.

In Chapter 4, Paper 3: Li, Y.; Dvořák, M.; Nesterenko, P. N.; Nuchtavorn, N.; Macka, M., High power deep UV-LEDs for analytical optical instrumentation. *Sensors and Actuators B: Chemical* **2018,** *255*, 1238-1243.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Mirek Macka and Pavel Nesterenko contributed to the idea, its formalisation and development. Miloš Dvořák and Nantana Nuchtavorn offered experimental assistance. All co-authors assisted with refinement and presentation.

In Chapter 4, Paper 4: Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Performance of a new 235 nm UV-LED-based on-capillary photometric detector. *Analytical Chemistry* **2016,** *88* (24), 12116-12121.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Mirek Macka, Pavel Nesterenko, Brett Paull and Roger Stanley contributed to the idea, its formalisation and development. All co-authors assisted with refinement and presentation.

In Chapter 4, Paper 5: Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., High sensitivity deep-UV LED-based z-cell photometric detector for capillary liquid chromatography. *Analytica Chimica Acta* **2018,** *1032*, 197-202.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Mirek Macka, Pavel Nesterenko, Brett Paull and Roger Stanley contributed to the idea, its formalisation and development. All co-authors assisted with refinement and presentation.

In Chapter 5, Paper 6: Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Nuchtavorn, N.; Krčmová, L. K.; Aufartová, J.; Macka, M., Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components. *Analytica Chimica Acta* **2015,** *896*, 166-176.

Yan Li was the primary author and conducted all the experiments, analysed data and wrote the manuscript. Mirek Macka and Pavel Nesterenko contributed to the idea, its formalisation and development. Miloš Dvořák, Nantana Nuchtavorn, Lenka Kujovská Krčmová and Jana Aufartová offered experimental assistance. All co-authors assisted with refinement and presentation.

We the undersigned agree with the above stated "proportion of work undertaken" for each of the above published peer-reviewed manuscripts contributing to this thesis:

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Date: 10 Oct 2018

List of Publications

Journal articles

1. Li, Y.; Pace, K.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Miniaturised electrically actuated high pressure injection valve for portable capillary liquid chromatography. *Talanta* **2018,** *180*, 32-35.

2. Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Comparison of cation-exchange capillary columns used for ion chromatographic separation of biogenic amines. *Journal of Chromatography A* **2018,** *1571*, 193-200.

3. Li, Y.; Dvořák, M.; Nesterenko, P. N.; Nuchtavorn, N.; Macka, M., High power deep UV-LEDs for analytical optical instrumentation. *Sensors and Actuators B: Chemical* **2018,** *255*, 1238-1243.

4. Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Performance of a new 235 nm UV-LED-based on-capillary photometric detector. *Analytical Chemistry* **2016,** *88* (24), 12116-12121. (**Front cover featured article**)

5. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., High sensitivity deep-UV LED-based z-cell photometric detector for capillary liquid chromatography. *Analytica Chimica Acta* **2018,** *1032*, 197-202.

6. Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Nuchtavorn, N.; Krčmová, L. K.; Aufartová, J.; Macka, M., Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components. *Analytica Chimica Acta* **2015,** *896*, 166-176.

Conference oral presentations

1. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Microfluidic breadboard approach to capillary liquid chromatography. In AMN/ISMM/APBCM/ANZNMF, Hobart, Australia, 2017.

2. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., The potential of under 250 nm deep UV-LEDs in chemical analysis. In 24th Annual RACI Analytical and Environmental Division R&D Topics Conference, Sydney, Australia, 2016.

3. Li, Y.; Müller, M. N.; Paull, B.; Nesterenko, P. N., Precise and fast determination of inorganic magnesium in coccolithophore calcite. In ACROSS International Symposium on Advances in Separation Science (ASASS2), Hobart, Australia, 2016.

4. Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Miniaturised modular capillary LC: Sensitive detection with UV-LED z-cell. In 16th Asia-Pacific International Symposium on Microscale Separations and Analysis (APCE2016), Johor Bahru, Malaysia, 2016.

5. Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Macka, M., Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components. In 23rd Annual RACI Analytical and Environmental Division R&D Topics Conference, Melbourne, Australia, 2015.

6. Li, Y.; Dvořák, M.; Nuchtavorn, N.; Nesterenko, P. N.; Stanley, R.; Macka, M., Portable LC Platforms: Concepts, Technical Issues, Applicability. In The International Chemical Congress of Pacific Basin Societies 2015, Honolulu, USA, 2015.

7. Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Aufartová, J.; Krčmová, L.; Macka, M., Portable capillary LC: A modular medium pressure system based on a microfluidic platform and designed with off-the-shelf components. In 20th International Symposium on Separation Sciences (ISSS), Prague, Czech Republic, 2014.

8. Li, Y.; Dvořák, M.; Krčmová, L.; Aufartová, J.; Nuchtavorn, N.; Nesterenko, P. N.; Stanley, R.; Macka, M., Portable moderate pressure capillary liquid chromatography based on a modular microfluidic system using off-the-shelf components. In 22nd Annual RACI Analytical and Environmental Division R&D Topics Conference, Adelaide, Australia, 2014.

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Conference poster presentations

1. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Microfluidic breadboard approach to miniaturised capillary liquid chromatography. In 17th Asia-Pacific International Symposium on Microscale Separation and Analysis, Shanghai, China, 2017.

2. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., High power deep UV-LEDs for analytical optical instrumentation. In 46th International Symposium on High Performance Liquid Phase Separations and Related Techniques, Jeju, Korea, 2017.

3. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., The potential of deep UV-LEDs below 250 nm in chemical analysis: 235 nm UV-LED photometric detection in capillary liquid chromatography, Pittcon, Chicago, USA, 2017.

4. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Miniaturised HPLC injection valve with potential for portable capillary LC, Pittcon, Chicago, USA, 2017.

5. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Miniaturised capillary LC: Sensitive UV-LED Z cell detection In ACROSS International Symposium on Advances in Separation Science (ASASS2), Hobart, Australia, 2016.

6. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Characterisation of three new ethylvinylbenzene-divinylbenzene based capillary columns

exhibiting ion-exchange and hydrophobic properties using biogenic neutral and charged solutes. In The 40th International Symposium on Capillary chromatography (ISCC), Riva del Garda, Italy, 2016.

7. Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., 255 nm and 280 nm UV-LED based photometric detection integrated with z cell for capillary LC. In The 40th International Symposium on Capillary chromatography (ISCC), Riva del Garda, Italy, 2016.

8. Li, Y.; Dvořák, M.; Nuchtavorn, N.; Nesterenko, P. N.; Stanley, R.; Macka, M., Portable capillary LC as a flexible design modular system based on a microfluidic platform and designed with off-the-shelf components. In 42th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC), Geneva, Switzerland, 2015.

9. Li, Y.; Dvořák, M.; Krčmová, L.; Aufartová, J.; Nuchtavorn, N.; Nesterenko, P. N.; Stanley, R.; Macka, M., Portable medium pressure capillary liquid chromatography based on a modular microfluidic system using off-the-shelf components. In Pittcon, New Orleans, USA, 2015.

10. Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Aufartová, J.; Krčmová, L.; Macka, M., Portable capillary LC: A modular medium pressure system based on a microfluidic platform and designed with off-the-shelf components. In 20th International Symposium on Separation Sciences (ISSS), Prague, Czech Republic, 2014.

Abstract

Underlying trends towards portable analytical instrumentation over the last few decades have not been equally reflected in the development of miniaturised and portable liquid chromatography (LC) instrumentation for rapid on-site or in-field measurements. Difficulties in accessing appropriate components have inhibited the design and fabrication of effective miniaturised LC systems. Additionally, the current small market for portable LC in comparison with some other miniaturised and portable analytical devices creates impediments to their commercial development. Therefore, the overall objective of this project has been to develop a new approach in designing a miniaturised portable LC based on a modular design that would harness the advantages of integrating different technologies offering flexibility in its configurations and use. In this process, it is necessary to explore available technologies, as well as develop new technologies, and integrate them in a miniaturised capillary LC system. This miniaturised capillary LC system with gradient elution capability was designed and the performance of each of the miniaturised component modules was tested and optimised. The design was based on a flexible modular system using primarily off-the-shelf components to ensure wide accessibility to the broader analytical community. A miniaturised capillary LC system was assembled on a breadboard, containing syringe pumps and switching valves, with an injection valve and on-capillary detectors, all controlled by a PC. Four miniaturised syringe pumps, with 5, 20 and 100 µL syringe options, formed the basis of the pumping system. Two pairs of pumps were used for each mobile phase to create gradient elution capability. The two pairs of syringe pumps were linked by two microfluidic switching valves and connected directly through a zero void volume cross-connector, thus providing a low hold-up volume for gradient formation. Sample was

injected by a 20 nL nano LC injection valve or a newly developed miniaturised LC injection valve. The electrically actuated valve features a very small size (65 x 19 x 19 mm) and light weight (33 g), and therefore can be easily integrated in a miniaturised modular capillary LC system suited for portable and/or field analysis. The internal volume of the injection valve was determined as 98 nL. The novel conical shape of the stator and rotor and the spring-loaded rotor performed well up to 32 MPa (4641 psi). A range of chromatographic columns suitable for operation under medium pressure range were selected and characterised. The range of selected columns covered anion-exchange, cation-exchange and reversed-phase separations. The retention of 15 biogenic amines and amino acids with three capillary cation-exchange columns IonPac CS19, CS12A and CS17 (250 x 0.4 mm ID, all from Thermo Fisher Scientific) with medium, medium low and ultra-low hydrophobicity and either carboxylic or mixed carboxylic/phosphonic acid functional groups was investigated. A new deep UV-LED-based photometric detection system for miniaturised LC was developed to complement the injector system. The development of the detection system included the study of new generation aluminium nitride (AIN) substrate based deep UV-LED, the development of a new 235 nm on-capillary photometric detector and the development of a deep UV Z-typed flow cell photometric detector, with an LED under 250 nm featured in analytical chemistry for the first time. The performance of the miniaturised LC system was evaluated theoretically and experimentally, including the maximum operating pressure, gradient mixing performance, and the performance of the detectors. The 5 µL syringe pump offered the best performance, with typical maximum operating pressures up to 11.4 ± 0.4 MPa and gradient pumping reproducibility of between 4% to 9% for gradients between 0.10 % s^{-1} and 0.33 % s^{-1}. The RSD (relative standard deviation) values of t_R and peak areas of 6 successive isocratic runs were 0.5%-0.7%

and 1.8%-2.8% for the separation of biogenic amines, respectively, and 0.1 %-0.2% and 2.1%-3.0% for the separation of cations, respectively. Test solutes of charged and uncharged dyes, and pharmaceuticals showed typical RSD values of 0.1 –0.2 % and 0.6 -1.0 % in isocratic mode and 1.2 – 4.6% and 3.2 – 6.4% in gradient mode, respectively for t_R and peak area reproducibility. The overall performance of the miniaturised modular LC was found in most parameters to be comparable or superior to most other reported miniaturised LC systems, with a clear potential to be portable. The potential of the platform for on-site analysis in future has been demonstrated.

Table of Contents

List of Abbreviations

AmpD	Amperometric detection
CE	Capillary electrophoresis
ECD	Electrochemical detection
FIA	Flow injection analysis
HPLC	High performance liquid chromatography
IEC	Ion exchange chromatography
k	Retention factor
LC	liquid chromatography
LED	Light emitting diode
LOD	Limit of detection
MPLC	Medium pressure liquid chromatography
RSD	Relative standard deviation
t_R	Retention time
UHPLC	Ultra high pressure liquid chromatography
UV	Ultraviolet
α	Separation factor
μTAS	Micro-total analysis systems

Chapter 1. Introduction

1.1 Overview

In modern world, miniaturisation is of significant importance in many fields of science, including engineering[1], medical science[2] and chemistry[3], in order to achieve better portability, less material, power and space consumption, and shorter analysis time. On one hand, the development of material science, information technology and mechanical engineering supports scientific instrumentation to be more portable and affordable. On the other hand, the advances in inexpensive mobile computers and smart phones provided the possibilities of using portable data acquisition systems for portable instrumentation. In the 1990s, the concepts of lab-on-a chip and micro-total analysis systems (μTAS) were introduced, leading an evolutionary trend of miniaturisation in analytical chemistry[4]. The progress of lab-on-a-chip and μTAS, together with other miniaturised classical technologies, provide a great opportunity to advance miniaturized chemistry devices that will enable the reduction of sample and reagent volume, rapid analysis and low operational cost.

For chemical analysis, errors in result introduced through sample decomposition and contamination during sample collection point and sample analysis is always a major concern, especially where human health is concerned. For portable analytical instruments, the main requirement is the ability to be applied at the sampling site[3]. In this case, a portable instrument normally requires high mobility, minimal consumables (less solvent or gas use and waste) and independent power-supply[3, 5]. In addition, there are several other additional requirements, including robustness, minimal sample preparation, rapid analysis and ease of operation.

Along with these requirements, many portable devices have been developed and commercialised, including mobile pH meters, electrochemical detectors and spectrometers. For flow based portable separation and analysis devices, various techniques have been developed, for example, using CE[6-7] and gas chromatography (GC)[8-9]. The general trend in miniaturization in chemistry has been towards miniaturized LC utilizing microfluidic chips for portable development. However, there are some inherent advantages of non-chip miniaturised fluidic systems, using capillary-based LC especially in terms of simplicity, robustness, affordability, and flexibility of design. Column-based LC has simple geometry and the technology of polyimide coated fused-silica capillary proven by decades of usage.

LC is one of the most commonly used analytical techniques and is applied in many areas of chemistry and biological science. Researchers have continued to make advances in its theory, practice, applications and instrumentation, especially throughout the last half century[10]. However, even though LC is widely used in laboratories, compared to other separation-based analytical instrumental techniques, LC has suffered a lack of development and application for in-field analysis where its flexibility for multiple analyses and high resolution power can be used in situations requiring more immediate answers such as for labile samples or for real time process control.

There are four main challenges in terms of advancing miniaturised LC systems: (1) The commonly used hardware in LC is bench-top scale, especially high pressure pumping systems and detection systems; (2) Supply and containment issues with organic mobile phase chemicals impedes delivery and creates disposal problems for in-field analysis; (3) Poor or unsatisfactory analytical performance and robustness of

scaled down (miniaturised) systems compared to precision laboratory-based (benchtop) LC; and (4) Designs for miniaturised LC systems that rely strongly on in-house fabricated components not widely available, thus causing a lack of accessibility to other users. These issues have resulted in difficulties accessing appropriate components thus inhibiting the design and fabrication of effective miniaturised LC systems. Additionally, the smaller market, in comparison with other portable devices, creates higher manufacturing costs for limited production series. Therefore, in this Chapter, we focus on each of the functional parts of a miniaturised LC system, in order to provide a clear overview of the developments and off-the-shelf availability of each of the components, which will help future researchers in their efforts designing miniaturised LC systems.

In contrast to on-chip LC, which has been many times reviewed in recent years[11-18], there have been only a few reviews targeting portable 'non-chip' LC instrumentation[5, 19-20]. This Chapter highlights recent status, factors in the design of miniaturised LC systems, and expected future developments. Special attention has been given to the miniaturized functional elements of miniaturised LC, which in the future would be applied for miniaturizing of portable-field LC system.

1.2 Design of portable and miniaturised LC instruments

1.2.1 Current commercial LC instruments

The design of bench-top LC instruments has been affected by the general trend towards miniaturization in instrumentation, reflected in the small size of some of the commercially-available LC devices. **Fig. 1.1** summarizes the size and weight of some widely-used commercially-available general-purpose LC instruments up to 2018.

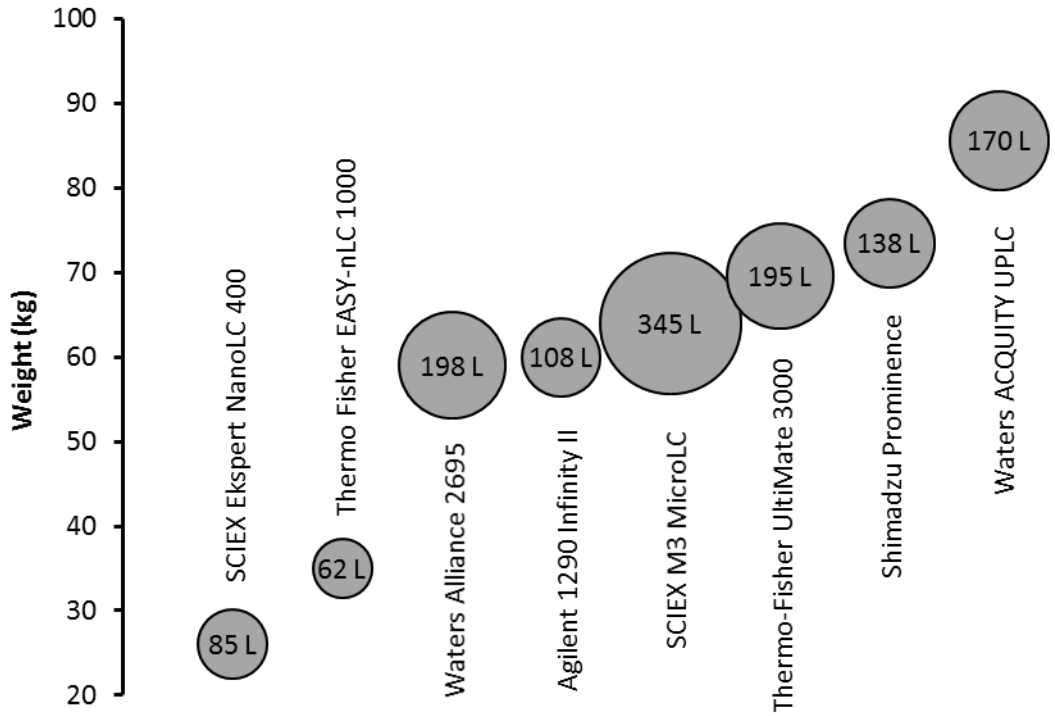

Fig. 1.1 Comparison of volume and weight of commercially available LC instruments. Space is calculated as effectively occupied by a box in which the instrument would fit. Information was sourced from vendor's specifications.

1.2.2 Miniaturised LC instrument design: definition and expectations

Features expected for a well-designed in-field portable instrument would include a compact design, mechanical rigidity and robustness to handling, and sufficient battery-powered operation time without interruptions due to re-charging. Low weight and small size are also needed for the instrument to be easily carried by one person. In addition, an integrated remote control and data acquisition system is desirable, especially for use in the case of long-time, multiple sampling analysis or analysis in a harsh environment. Therefore, in this thesis, we defined a "portable" instrument as a battery-powered device operated independently of mains power with a maximum weight of 10 kg. Those small size and light weight LC system not yet powered by a

battery will be here termed “miniaturised”. In addition, in comparison with the current sophisticated bench-top instruments, operation of a portable instrument should be simplified to allow use by a non-expert or an operator with little experience.

1.2.3 Commercialisation towards miniaturised LCs

Since HPLC was introduced in the mid-1960s, no portable or miniaturised LC system was manufactured until the first system was released in 1983 by Baram *et al.*[21]. This so-called OB’-4 or MiLiChrom micro-column LC was actually a lab-station portable system, as the weight was 42 kg and a regular line power supply was needed. This system executed the idea of using miniaturised micro-columns in a portable LC to minimise the solvent and sample consumption. Although, this device will not satisfy today’s terminology of portability regarding the dimension and weight, it still should be noted that this portable LC system was then commercially available on market for more than 10 years. Over 5000 machines have been sold (records till 1996)[22], which evidences that the market demand for portable LC instrumentation was already significant two decades ago.

Electrochemical detection (ECD) was first introduced into portable LC in 1986 by Otagawa *et al.*[23-24] for the determination of primary aromatic amines. Although the pumping system of this device was battery-driven, the detection system was a conventional potentiostat requiring a regular line power supply. Then, over the next decade, some studies towards in-field analyses were reported using MiLiChrom micro-column LC developed by Baram[25-27], but these developments cannot be regarded as an instrumentation breakthrough for portable LC. In 1996, after the previous version of MiLiChrom was updated to be “MiLiChrom-4”, in an improved version released by

Baram[22]. This new portable LC system weighed 14 kg and was driven by two syringe pumps, which enabled gradient elution mode. Like MiLiChrom, this instrument was designed for use in mobile laboratories where regular line power is available. In 1998, another portable LC system called "Minichrom" was designed by Solvalub Technologies[28]. This 9.5 kg system was battery powered (12 V DC), and mobile phase was delivered by two reciprocating pumps. The flow rate range for each pump was 0.1 – 2.5 mL, which enabled the use of micro-columns. However, even though these two portable LC systems were aimed to be commercialized, there is no evidence showing that they were commercially produced in significant numbers or widely used. The reason could be that the dimension and weight of commercial LC systems had decreased substantially through the decade (**Fig. 1.1**), and therefore these new "portable LC" devices, which weighed around 10 kg, may have lost their advantage of portability. In 2003, a commercial isocratic portable HPLC system called "CHANCE" that weighed 3.5 kg was introduced[29], which became the smallest HPLC at that time. This system supported a mobile phase flow rate range from 1 μL min^{-1} to 10 mL min^{-1}. In terms of weight and size, it could satisfy the expectation of a portable LC system. However, based on the authors' own experience, the eluent delivery system may have suffered a significant level of pulsations at low flow rates (<10 μL min^{-1}), which made this portable LC system not well suited for use with capillary columns. Recently, Lee's group developed a battery-operated nano-flow portable LC system thoroughly demonstrated in a series of studies[30-32], with excellent isocratic and gradient separation performance (**Fig. 1.2**). The UHPLC system with integrated sample injector offered a satisfactory separation performance and excellent detection performance using a UV-light-emitting diode (LED) photometric detector[33].

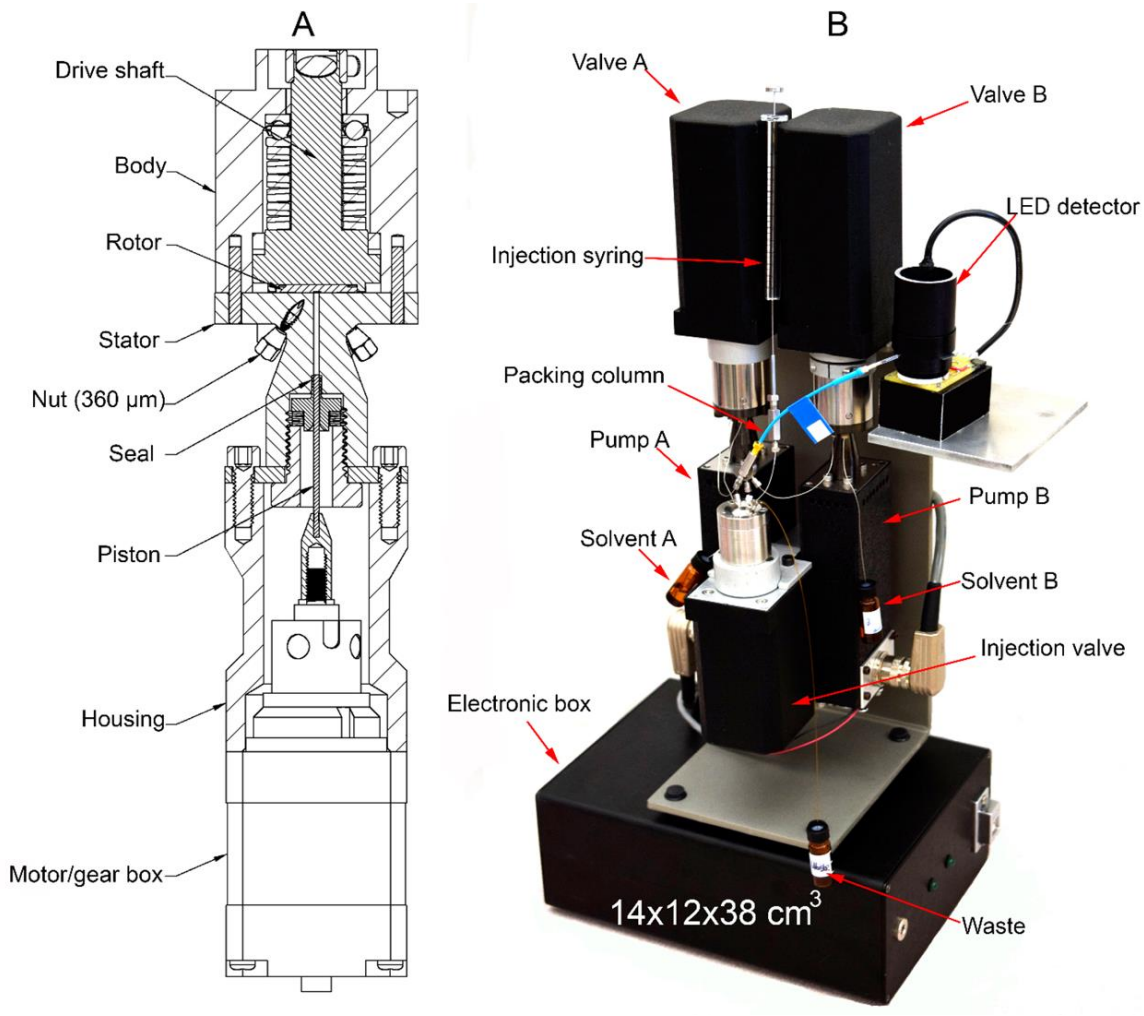

Fig. 1.2 A. Schematic drawing of a single nanoflow pump; **B**. Photograph of the portable nanoflow ultra high pressure liquid chromatography (UHPLC) with LED detector. The system weighed 5.9 kg or 4.5 kg without a controller and could hold up to 110 MPa (16000 psi) of pressure[32]. Reprinted with permission from American Chemical Society.

1.2.4 Research-based miniaturised and portable LCs

Apart from those portable and miniaturised LC system aimed at commercialisation, many studies in laboratory-built portable or miniaturised LC systems have been published, including ion-exchange chromatography (IEC)[25, 34-38], immunoextraction-reversed-phase LC[39], immunoaffinity chromatography[40] and reversed phase LC[41]. Several portable or miniaturised IEC systems were consecutively reported. One

possible reason for this was that the system pressure requirement for IEC is lower than that for RPLC, and therefore less challenged for the development of a suitable pumping system. A USB power-driven portable IEC was introduced by Kiplagat *et al.*[37], which with a weight under 1 kg is currently the smallest column-based portable LC in the world (**Fig. 1.3**). The pumping system is a miniaturised air pressure cylinder, providing only 15 MPa (100 psi) maximum pressure. With laptop USB power, this portable LC can be operated for more than 8 hours. A solar panel powered "portable" IEC was reported by Elkin[38]. Despite its heavy weight (27.5 kg), this IEC was the first in-field LC system with a photovoltaic power supply. This was also a fully automatic, sophisticated LC system, comprising an eluent reuse system, autosampler, sample preconcentration system and suppressor. The system provided sample analysis for a continuous 27 days, with a limit of detection (LOD) for phosphate of 10 µg L^{-1} (10 ppb).

In 2015, our group reported a miniaturised medium pressure capillary LC system with modular flexible open platform design[41]. This miniaturised LC system was based on off-the-shelf components, leading to the potential for wide accessibility. It is important to note that some conventional bench-top LC components had become relatively small, but those specified as "fully portable LC" systems are still rare. Furthermore, the dominant focus in research on chip-based separations may have detracted from the importance and further potential development of non-chip portable column-based LC.

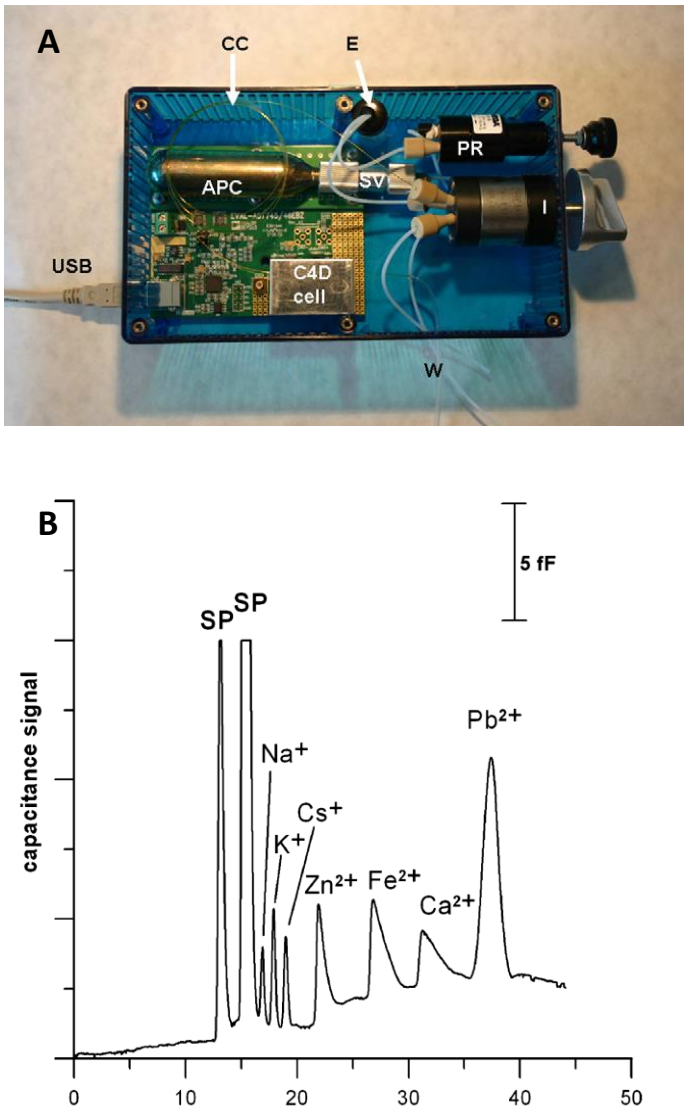

Fig. 1.3. A. Photograph of pressure based portable IEC instrument with integrated miniaturised C^4D. APC: air pressure cylinder, CC: capillary column, E: eluent reservoir, I: injection valve, PR: pressure regulator valve, SV: stop valve. **B.** chromatogram of a separation of alkali, alkaline earth and transition metal cations by the USB driven portable LC with integrated non-suppressed C^4D[37]. Reprinted with permission from Elsevier.

1.3 Functional components of miniaturised LC systems

1.3.1 Columns

The format and size of the LC column will determine to a large degree the design and portability of the whole LC system. Compared to other components of an LC system,

the physical dimension of an analytical LC column body is relatively small. Therefore, the focus of LC column miniaturisation, unlike other LC system components that aim to reduce the physical demission and weight, is to decrease the inner diameter (i.d.). According to the i.d. and operation flow rate range, miniaturised LC columns can be categorised into micro-columns (≥0.5 ≤1.0 mm i.d.), capillary columns (≥0.1 <0.5 mm i.d.) and nano-columns (<0.1 mm i.d.)[42]. A smaller column i.d. results in a reduction in mobile phase and sample volume consumption by up to three orders of magnitude (due to inverse proportionate dependence of flow linear velocity on column cross-section or square of i.d.). Lower flow rates also have an advantage regarding coupling to detection using mass spectrometry (MS). Additionally, miniaturised LC columns of smaller i.d. can lead to increase in column efficiency due to lower dispersion and flow resistance to mass transfer[43-44].

The history of LC column miniaturisation is longer than the miniaturisation LC of instrumentation. Research in LC column miniaturisation became popular in the 70s and 80s. Early progress in research on miniaturised LC columns was reflected in several reviews[45-47]. The academic interest in miniaturised LC columns declined somewhat in the early 90s, in part due to weaker detection signals due to lower injection volumes[48]. Miniaturised LC columns regained interests since 2000s due to the demand from several biology-oriented research areas (e.g. metabolomics research), where only limited amount of sample is often available. Since then, miniaturised LC columns and capillary/nano benchtop LC systems started to be increasingly commercialised. Miniaturised LC columns development has been thoroughly reviewed[48-51], and the instrumentation for miniaturised LC column was also reviewed recently[52]. In this Section, we focus on the overall trends in miniaturisation of LC

instrumentation, and therefore miniaturised LC column stationary phase development, which is a relatively independent area, is not included. Instead, the synergy of miniaturised LC columns and miniaturised LC systems and the trend of incorporating miniaturised LC columns in miniaturised LC systems will be discussed.

As stated before, miniaturised LC column development is relatively independent to the miniaturisation of the LC instrumentation. In fact, in the literature the term "LC miniaturisation" sometimes only indicates "miniaturisation of LC columns"[48]. Because of the different timelines and aims in the developments of miniaturised columns and of miniaturised LC systems, in the early stages they became somewhat mismatched and independent of each other. Miniaturised LC columns were often developed targeting bench-top capillary/nano HPLC systems, instead of miniaturised LC systems. In contrast, early miniaturised LC systems usually used conventional size packed columns due to their availability[22-23, 28]. However, low solvent consumption is extremely desirable for miniaturised LC systems. Since miniaturised LC columns became increasingly commercially available with time, the trend now in miniaturised LC systems is to use miniaturised LC columns. **Fig. 1.4** clearly shows that the synergy trend of miniaturised LC columns and newly reported miniaturised LC systems (including those with non-portable detectors) has been increasing, and after 2010, 90% of the newly reported miniaturised LC systems support miniaturised LC columns.

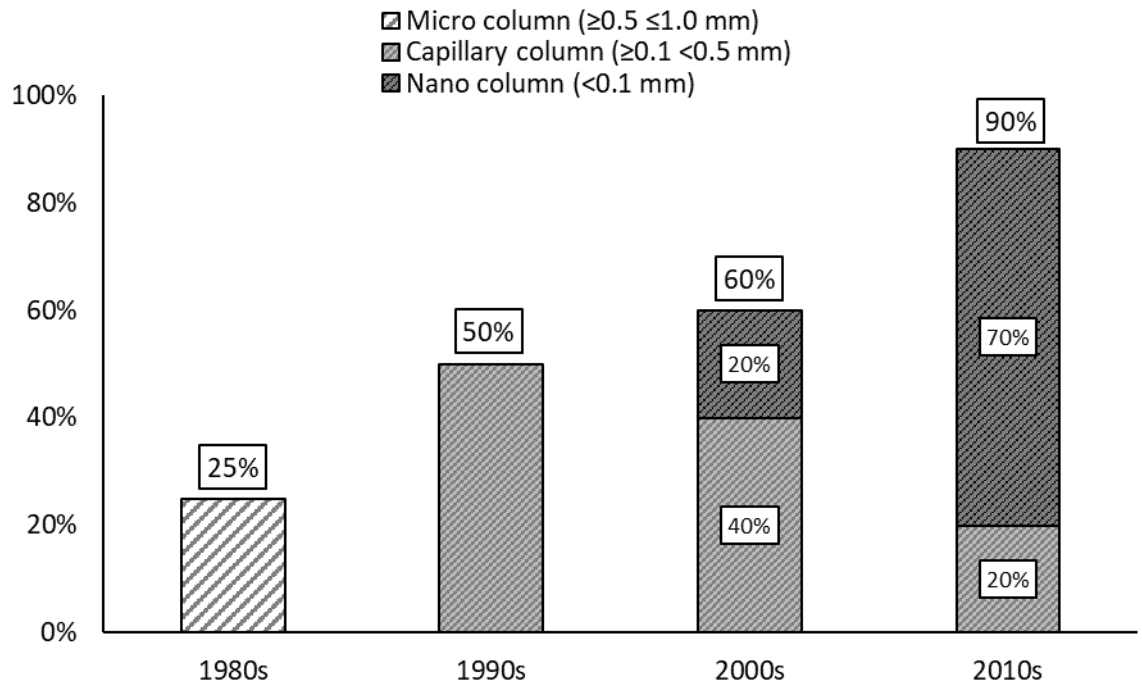

Fig. 1.4 The percentage of newly reported miniaturised LC systems (including those with non-portable detector) that support miniaturised columns during each decade. For the systems that support a wide range of column, the most miniaturised column format was selected. (The % numbers are calculated from the numbers of miniaturised LC systems reported in scientific papers or commercialised).

In order to achieve rapid and high efficiency separations, on-going research on miniaturised LC columns increasingly focus on UHPLC capillary columns[48, 51]. The increased operating pressures required for these UHPLC capillary columns brings challenges in the miniaturised LC instrumentation development, and in particularly for the pumping system. Though there are recently introduced miniaturised pumps for UHPLC (**Section 1.3.3**), most of them are still at a development stage and need a wider market and user feedback before becoming more widely adopted. More importantly, the most commonly used high pressure dual-piston pump has still not been

miniaturised with a satisfactory performance[29]. Therefore, the medium pressure miniaturised LC columns (e.g. Chromolith from EMD Millipore) will continue to be desirable for miniaturised LC systems before miniaturised UHPLC pumps achieve a technology breakthrough.

1.3.2 Injection system

Early HPLC systems that only operated under low-pressures widely used septum-type injectors. However, these types of injectors lack reliability and user-friendliness[53], and are also not suited for automation[54]. Stop-flow injection was introduced by Waters in their U6K in 1975[55]. As the injection technique name suggests, the pump flow must be stopped while sample injection is made. In comparison to septum-type injectors, stop-flow injection allows easy and steady injection into a relative high-pressure system. A Stop-flow injection system was integrated into the portable nano HPLC solvent delivery systems recently reported by Lee's group[30-31]. Six-port continuous-flow injectors, introduced in 1976, provided uninterrupted high pressure flow injection, and eventually became the industry standard[53]. The continuous-flow injectors are normally an individual component that can be modular, therefore they have been used widely in miniaturised LC systems[23, 28-29, 41, 56].

Low solvent consumption is naturally desirable, and for miniaturised LC systems it is even more crucial. Miniaturised LC systems were normally designed as capillary/nano LC form, using low injection volumes. For nano LC systems, where less than 20 nL injection volume is needed, the only available continuous-flow injectors are those with an integrated internal sample loop. However, they lack flexibility, as changing the sample injection volume requires a change to the entire injection valve (or a part of it).

Therefore, for capillary LC systems, where the required injection volume is more than 100 nL, a continuous-flow injector using an external sample loop is the best option[19].

The general opinion is that the size and weight of standard HPLC injectors are small enough so that there are no principal changes needed for miniaturised LC injectors[5]. Indeed, a 1 kg standard stainless steel based injector does not have as strong impact on the overall LC system or on early stage miniaturised LC systems, in comparison to relatively bulky solvent delivery and detection systems. Based on this view, there is lack of significant efforts towards miniaturisation of HPLC injection valves[49].

However, the trend towards miniaturisation makes the overall scale of miniaturised LC smaller and lighter. For example, in 2015, our research group reported a portable modular medium pressure capillary LC system[41]. This system was based on off-the-shelf components assembled on a breadboard of a commercially available flexible microfluidic platform. Though almost all the components used in this system were miniaturised, the sample introduction system was a standard stainless-steel injection valve. However, this was the only commercially available capillary/nano injection valve option on the market at that time. The weight of the standard injection valve itself took some 66% of the entire LC system (0.86 kg out of 1.30 kg). Therefore, a standard injector became one of the bottlenecks in the miniaturisation of LC. This year, a new miniaturised high pressure 6-port injector was reported and applied in a miniaturised LC system[57] (**Fig. 1.5**). This injection valve features a very small size (65 x 19 x 19 mm) and light weight (33 g), making it currently the smallest and lightest commercially available high pressure LC injection valve. The result shows this injection valve gave a comparable performance to a standard 6-port injector.

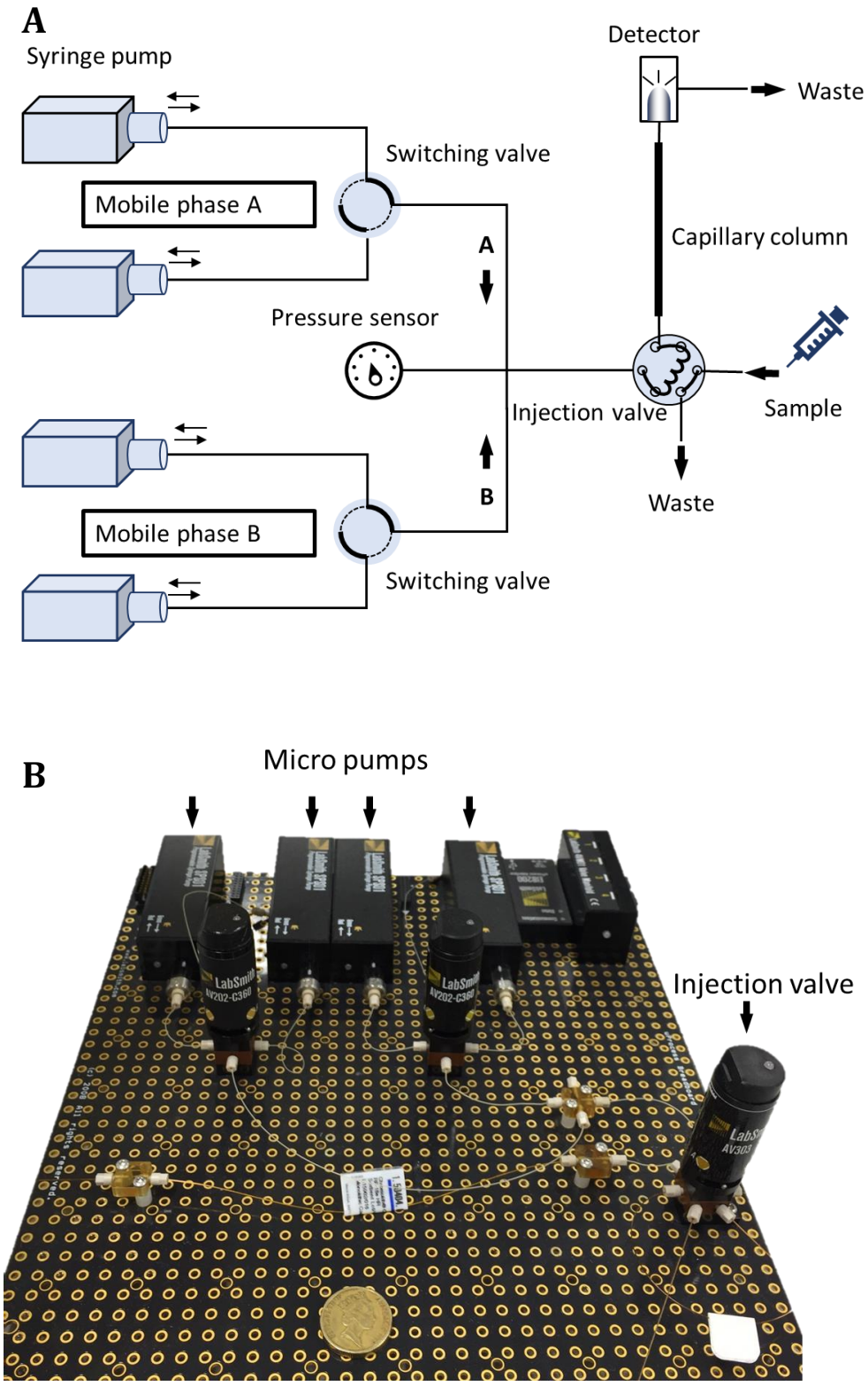

Fig. 1.5 The schematic (**A**) and photograph (**B**) of the miniaturised injection valve and miniaturised LC system[57]. Reprinted with permission from Elsevier.

1.3.3 Solvent delivery system

The solvent delivery system is one of the most crucial parts for an HPLC system, as it determines the maximum pressure of operation, the precision and accuracy of the fluid flow and the flow rate scale. Since the column particle size (i.d.) decreased dramatically over the past decades to achieve higher column efficiency, the desirable maximum operating pressure for HPLC pumps has increased significantly up to 140 MPa (20000 psi) for modern UHPLC systems[10, 53]. However, regardless of the increased pressure of operation, the basic requirements for HPLC pump remained the same. The high pressure pump options for HPLC include reciprocating pump[54], syringe pumps[54] and electro-osmotic pumps[58-60]. For miniaturised LC systems, some low pressure pumps were also used, such as direct piston pumps[23] and peristaltic pumps[36]. Due to the applicability of and trends in HPLC development, in this Section we will only focus on high pressure HPLC pumps.

Reciprocating pumps are the predominant form for commercial benchtop HPLC systems, as they can operate under extremely high pressures (>150 MPa), they are robust and they are easy to use[61]. Syringe pumps used to be a competitor of reciprocating pumps for HPLC system before 1980s. However, in 1975, Michel *et al.*[62] raised the issue that solvent compressibility, caused by syringe pumps, results in long stabilisation times before a steady-state condition could be achieved. Following this report, the applicability of syringe pumps for HPLC was hotly discussed[63-65]. These studies concluded that a sophisticated design, with integrated pressure controllers, is needed for syringe based pumping system, especially for standard HPLC systems. The increased complexity of syringe based pumping systems, together with the disadvantage of refilling eluent, deflected the market attention towards other solutions.

In modern HPLC, the Waters M-6000 reciprocating pump[53] become the "gold standard", which led to the reciprocating type pump dominating most, if not all, of the commercial standard HPLC systems.

Since the 1980s, the prospects of HPLC moving from conventional bench top scale into the portable capillary domain has gained considerable attention[50]. However, the reciprocating pump systems capable of offering nL - μL min^{-1} flow rates were rare on the market during that time (1980s-1990s) as well as expensive[66], and the pulsations at low flow rates were significant[5]. The bulky dimensions of reciprocating pumps also make them, in principle, impractical to be applied in miniaturised LC systems. Although some miniaturised LC systems still utilised reciprocating pumps during the 1990s[28] and early 2000s[29, 39], these systems were usually relatively large for miniaturised LC[28, 39], or they only supported conventional columns[28]. These miniaturised LC systems were all discontinued, and there was no newer version further released.

The difficulty of implementing reciprocating pumps in portable capillary LC led to interest in using syringe pumps. Early reports found that the liquid compressibility issue in syringe pumps become marginal for HPLC when the syringe volume (mL) to piston flow (mL min^{-1}) ratio is less than 100 mins (stabilisation time < 5 mins at 9.3 MPa)[62]. Therefore, assuming the flow rate in a capillary LC is 10 μL min^{-1}, to eliminate the liquid compressibility issue, the syringe volume should be less than 1 mL. On the other hand, to be practically useful in a miniaturised LC system, the syringe volume of a syringe pump should be small (<5 mL) to fulfil a compact size. Both factors match well with each other, suggesting that syringe pumps are suitable for portable capillary LC. Some studies[66-67] have been conducted on the development of miniaturised syringe

pumps and syringe pumps have become the choice of pumping systems of some miniaturised and portable LC systems[21-22, 30-31, 34, 41]. Among these systems, two of the recent miniaturised LC pumping systems can be highlighted. The first one is used in a hand portable LC system reported by Lee's group (**Fig. 1.2**)[30-32]. This pump can provide up to 110.3 MPa (16,000 psi) pressure with accuracy > 99.9% at flow rates 0.3-6 µL min^{-1}, which makes it an UHPLC pump. The second pumping system reported by our group, uses multiple miniaturised micro-syringe pumps assembled on a microfluidic breadboard in a LEGO-like fashion (**Fig. 1.5**)[41, 57]. Two pairs of micro-pumps were used for each mobile phase to enable continuous pumping, as well as gradient elution capability, while additional micro-syringe pumps could be used for other functions such as sampling and delivering post-column reagent. The maximum pressure of these micro-syringe pumps was up to 11.5 MPa (1523 psi), so they could not be classified as an HPLC system, but the maximum pressure was enough for use with low pressure, especially monolithic, columns.

High pressure electro-osmotic pumping (EOP) has been shown to deliver pulseless nanoflow up to 120 MPa[58], and was applied in nano HPLC for isocratic separations[58, 68], as well as for gradient separations[60]. One of the advantages of EOP is that the pressure output of an EOP system can be proportionally increased by the addition of more pump units. From these studies, EOP seems to be an ideal candidate for miniaturised LC, as it is compact, pulseless and able to deliver low flow rates at high pressure. However, the major disadvantages of implementing EOPs in HPLC are its complex operation, and the flow rates which vary with different backpressures of different columns. In addition, there is no commercially available high pressure EOP on the market yet. Given the fabrication of a high pressure EOP is technically

sophisticated, the use of EOPs in HPLC is thus limited to a few research groups. There has been only one miniaturised LC reported using EOP[56] until now. However, this system is a low-pressure LC, and the EOP could only deliver eluent under maximal 1 MPa pressure. Undoubtedly, more investigations should be conducted to evaluate the applicability of high pressure EOP for miniaturised LC.

1.3.4 Detection technology

The detection system requirements for miniaturised LCs include small size, light weight, low power-consumption and high sensitivity. It is understandable that miniaturisation and low-power consumption are the essential elements for miniaturised LC detection systems. We particularly emphasise the importance of high sensitivity in miniaturised LCs detection system based on following criteria: (1) To maintain the simplicity, miniaturised LC designs normally do not include a pre-concentration component. Therefore, high sensitivity is highly demanded, especially for in-field analysis. (2) Miniaturised LCs are normally in capillary/nano LC form, with nano level sample injection volumes. The 100-10000 times lower volume of injected analytes result in significant orders of magnitude decreases in signal sensitivity which must be compensated through a correspondingly higher sensitivity detector. As a consequence, although the detection technologies employed in conventional LCs possess a large diversity (including refractive index, fluorescence, chemiluminescence, evaporative light-scattering, UV-absorption, electrochemistry and mass spectrometry), only ECD and UV-absorption detectors have been integrated with portable LCs. Mass spectrometry (MS) is an ideal detector for LC, however, the performance and the size of miniaturised MS is still not satisfactory for an integrated miniaturised LC-MS.

ECD, including amperometric detection (AmpD) and contactless conductivity detection (C^4D), has been successfully miniaturised and applied in numerous portable flow analysis devices. In fact, in a portable CE system, because of the ease of miniaturisation, ECD is one of the most commonly used detection techniques[69]. However, ECD is normally sensitive to eluent composition, temperature and flow rate, while for a portable LC using miniaturised pumps for in-field analysis, flow rate fluctuation and changes of temperature are often inevitable. More importantly, without a suppressor, conductometric ECD is not applicable to gradient elution. The first LC that applied ECD was one of the earliest portable LCs introduced in 1986[23]. The detector cell was a highly miniaturised thin-layer transducer cell, containing a glassy carbon working electrode and an Ag/AgCl reference electrode, while the cell potential was still controlled by a commercial potentiostat. The LOD of this ECD was under µg L^{-1} (ppb) level for polycyclic aromatic amines. However, as expected, the baseline signal was affected by eluent composition, the change of flow rates and temperature. In 2010, an USB driven portable LC with an integrated C^4D was reported by Kiplagat *et al.*[37] (**Fig. 1.3**). This C^4D cell was designed using an evaluation board (EZ AD7746EB; 5 V; 32 kHz capacitance measuring rate) with electrode distance of 0.5 mm. As the detector operating voltage was only 5 V, a grounding plane between the electrodes was not included. The detector offered an LOD of 18.4 µg L^{-1} (ppb) for Na^+. Following this, a few portable LCs with ECD for ion analysis[37-38, 56] were reported, although they only supported isocratic separation under low or medium pressures (< 34.5 bar (500 psi)). However, ECD, with its excellent sensitivity and unique advantage for ion analysis, is likely to gain more attention once the performance of miniaturised high pressure pumps improves. Also, the upcoming breakthrough of miniaturisation

of suppressors[70] will enable ECD to be used under gradient elution, which will dramatically increase the applicability of ECD.

UV absorption detection is almost universal in most LC separations[53]. UV detection is considered a robust, easy to use and low-cost technique, although its sensitivity and selectivity is lower than for ECD. A standard UV detector consists of a light source, wavelength selector, flow cell and detector to measure light passing through the flow cell. For early miniaturised LCs, due to overall dimension of the LC and the available technology, the detection system was not the key element for the whole LC system miniaturisation. The first portable LC used a standard UV detection system, where a deuterium lamp and diffraction grating mirror were used[21-22]. Fixed wavelength UV detection was then introduced to portable LC[28]. The fixed length detector had improved compactivity and sensitivity, while changeable lamps for different wavelengths offered a broad applicability of the LC system. The LOD of the portable LC was 200 µg L^{-1} (ppb) and 15 µg L^{-1} (ppb) for phenol and anthracene, respectively.

The introduction of LEDs had a revolutionary impact for optical detection and especially its miniaturisation. LEDs offer small dimension, low weight, low cost, low power consumption and monochromaticity of the emission wavelength, which are all crucial for design of miniaturised optical detection devices. A number of LED absorption detectors for CE or LC have been reported, first with red, then green and later blue LEDs[71]. The first deep UV-LED absorption detector was introduced by Schmid *et al.*[72] for a standard HPLC system. Due to the low LED light intensity, the detector LOD was about 10 time higher than for a standard LC detector. Since then, many studies of absorption detectors based on deep UV-LEDs have been reported, including in CE[73], and LC[74-76]. For portable LCs, in 2015 Lee's group introduced an

on-column 260 nm LED detector. This detector implemented focusing lenses and a slit to increase the light intensity and reduce the stray light, which resulted in an extremely low noise level (4.4 μAU) and excellent LOD (7.6 μg L^{-1} (ppb) for sodium anthraquinone-2-sulfonate)[33]. This detector was then used on the portable LC developed in Lee's group[31-32] (**Fig. 1.6**). In the same year, our group reported an on-capillary 235 nm LED detector, which was the lowest wavelength LED used in an optical detector[77]. This detector demonstrated the first direct LED based detection of nitrite and nitrate, and was then applied on the miniaturised LC introduced by our group[41] (**Fig. 1.3**). This year, Lee's group reported a dual-wavelength LED-based UV absorption detector for nano-flow capillary LC[78] (**Fig. 1.6**). This single unit of the LED detector is only 30 g, with the linearity across 3 orders of magnitude from nM range. Two single units can be assembled to a dual wavelength detection system with 255 nm and 275 nm LEDs.

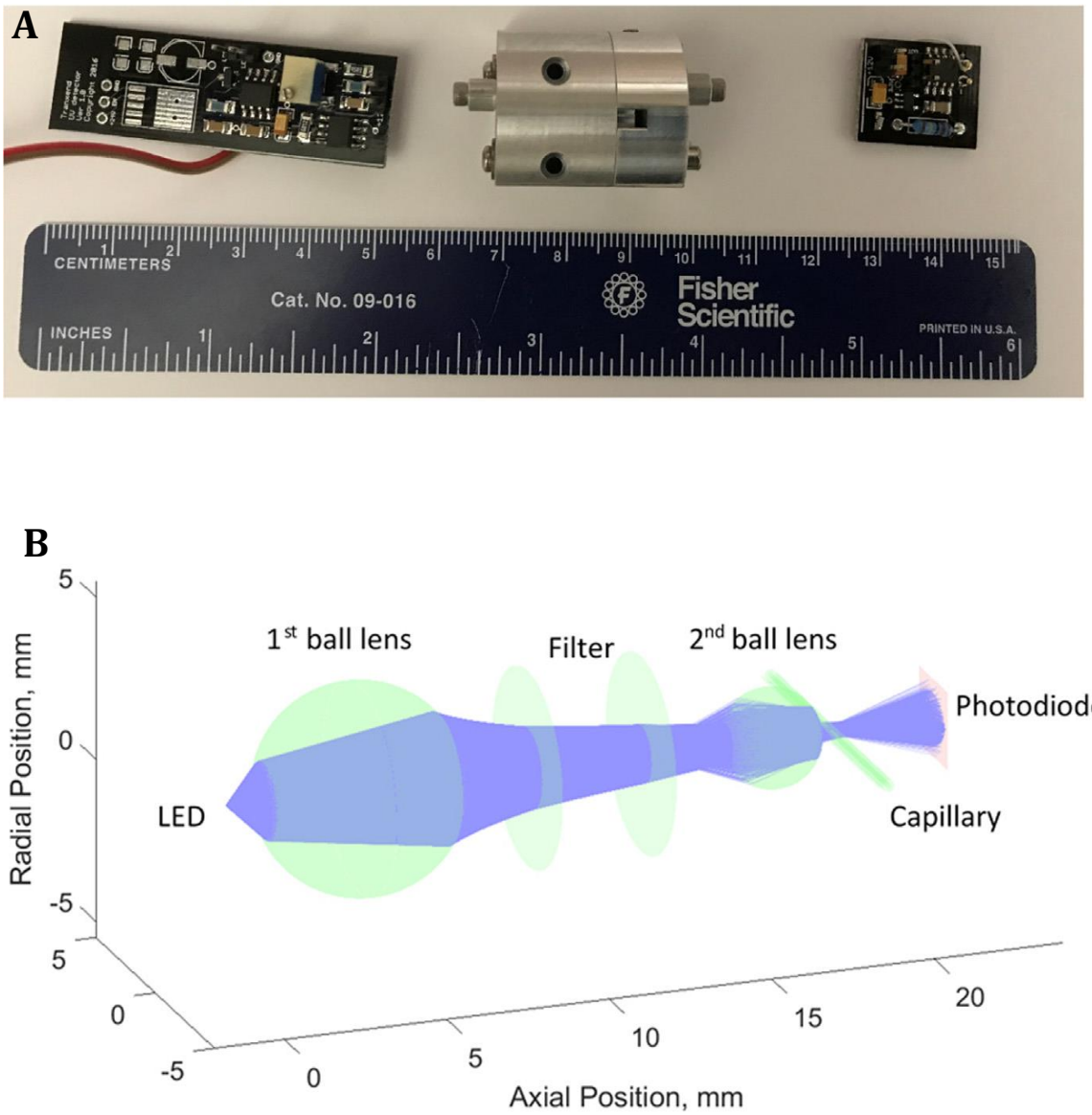

Fig. 1.6. A. photograph showing the sizes of the new detector and the PCBs for LED driver (left) and photo diode (right)**. B.** 3D ray tracing model of the new detector[78]. Reprinted with permission from Elsevier.

The effective path length of on-capillary/column absorption detector is limited by the capillary/column i.d., normally around 100 µm. To further improve the detection sensitivity, a deep UV-LED detector with a z-shaped nano flow cell was recently developed, with a 100 times increased path length, resulting in the detection sensitivity increasing 50 times[79]. The performance of this miniaturised LED detector was shown to be comparable and superior to the conventional benchtop UV detectors. High-power

deep UV LED's with a higher monochromaticity (lower parasitic emission in the visible spectral range) are already commercially available[80]. In addition, UV-LED technology is still advancing rapidly, so commercial deep UV LEDs will become available at lower wavelengths, with stronger optical intensity and longer life times. All these factors will provide deep UV-LEDs with a greater applicability in absorption detection.

1.4 Concluding remarks

Miniaturization influences all areas of science and technology, impacting positively on the size and the potential portability of existing LC instrumentation. In the field of analytical and separation sciences, and specifically for LC, a substantial source of progress in miniaturization will be from general progress in technology (i.e. reduced component sizes including those of separation columns, dimensions of detection cells and operating electronics). This will also have positive influence on the development of miniaturised/portable instrumentation.

The trends in portable and miniaturised LC, miniaturization efforts have focused on microfluidic chip-based LC. However, numerous in-house designed miniaturised/portable LC instruments have been reported, showing the importance of portable, column-based LC research. It is important to emphasize that column-based LC has distinct advantages, namely, simplicity, affordability, flexibility of design and robustness based on the ultimately simple geometry and technology of polyimide coated fused-silica capillary proven by decades of usage.

Miniaturization of LC pumps, as the central parts of LC design, is a key to successful miniaturised/portable LC development. High pressure reciprocating pumps are the most commonly used pumps for benchtop LC, but other eluent delivery techniques, in particular high pressure syringe pumps and EOP hold promise for future developments in miniaturised/portable LC.

As the most commonly used high pressure reciprocating pumps still have not been miniaturised with a satisfactory performance, medium pressure miniaturised LC columns (e.g. Chromolith from EMD Millipore) are still be desirable for miniaturised LC systems until miniaturised UHPLC pumps achieve a technical breakthrough and become widely available.

From the point of view of suitable detection for miniaturised/portable LC, ECD has the advantage of compatibility with miniaturization and portable instrumentation. Miniaturized, low-cost light sources (e.g., high-power LEDs and LDs) are very efficient, inexpensive alternatives to traditional light sources (e.g., laser modules for LIF) so LEDs and LDs are also likely to find their way into miniaturised/portable LC.

The drive for miniaturised and portable LC comes from the needs in many in-field or on-site application areas utilizing analysis techniques, for example real-time process control or monitoring, and modern technology-enabled traditional techniques. The areas include environmental monitoring, quality control and quality assurance lab and security screening (*e.g.* airport security). Low-cost system off-the-shelf approach may also have an excellent educational value especially given the modular, flexible and low-cost design philosophy. These application-driven needs for miniaturization combine with the enabling developments in technology (e.g., fuel-cell technology,

materials science, electronic semi-conductor, power sources and other components, and miniature electromagnetic actuators). Developments in these areas drive and enable future developments in miniaturization of components and integrated systems. The enabling technologies are continually being developed and therefore available for further improvements in the miniaturised/portable LC devices.

1.5 Aims of the project

The overall objective of this project has been to understand and integrate some of the available technologies, as well as develop new technologies, in order to develop a miniaturised capillary LC system.

The specific aims of the project were to:

1. Develop the design and explore the advantages of a novel miniaturised LC platform based on off-the-shelf components. The basis of the system was a modular microfluidic platform with the components assembled on a breadboard. The investigation within this project focused on the performance of each miniaturised functional components, including sample injection and solvent delivery system.

2. Investigate options of suitable narrow-bore or capillary chromatographic columns. It was necessary to find columns with small dimensions, high separation efficiency, minimum sample volume needs and very low solvent consumption. Additionally, the column needed to be capable of operation under medium backpressure.

3. Investigate and develop novel deep UV-LED photometric detectors for miniaturised LC systems. LEDs can be very suitable light sources due to their robustness, small size, lack of need for a monochromator, low current consumption, and relatively low price.

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Chapter 2. Fluidic system

2.1 Miniaturised electrically actuated high pressure injection valve for portable capillary LC

*All of this research contained in this chapter has been published as Li, Y.; Pace, K.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Miniaturised electrically actuated high pressure injection valve for portable capillary liquid chromatography. *Talanta* **2018,** *180*, 32-35.

Supplementary Information (SI)

Miniaturised electrically actuated high pressure injection valve for portable capillary liquid chromatography

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1. Experimental

1.1 Chemicals and reagents

Analytical or higher purity grade reagents and deionised water (Millipore, Bedford, MA, USA) were used for preparing all solutions. Methanesulfonic (MSA) acid was generated by an ICS-3000 Eluent Generator (Thermo Scientific Dionex, Sunnyvale, CA, USA). Uracil, sodium chloride, lithium chloride, ammonium chloride, potassium chloride, L-DOPA, phenylephrine, norfenefrine, 5 hydroxytryptophan, dimethylamine and trimethylamine were purchased from Sigma-Aldrich (St. Louis, MO, USA).

1.2 Methods and procedures

The internal volume measurement was through the injection of uracil solution (1 mg L^{-1} in deionised water) using different volume of external sample loop. The uracil was then detected using a UV detector (254 nm). The linear dependence between the peak area and the volume of external sample loop was then obtained. Injection carry-over was studied by first injecting a concentrated uracil solution (500 mg L^{-1} in deionised water). The injection valve was then thoroughly cleaned by purging with deionised water and a blank injection (deionised water) was made with carry over detected using a UV detector (254 nm). These experiments were repeated five times.

Injection performance under different backpressures was studied with sample injection of sodium chloride (10 mg L^{-1}). The sample was detected using a C^4D detector (voltage -12 dB, gain 50%) at the shortest possible distance from the injection valve (55 mm i.d. 100 µm capillary). The solvent used was deionised water. Temperature: 20 °C. Flow rate 10 µL min^{-1}. Two restrictors made by polyimide coated fused silica capillary were used: medium pressure restrictor (10 µm x 33 mm) and low pressure restrictor (25 µm x 90 mm). The detailed experimental setup is illustrated in SI, **Fig. S4 A**, **B** and **C**.

For the maximum operating pressure study, a high pressure restrictor made by polyimide coated fused silica capillary was used (10 µm x 44 mm) and all experiments were performed at 20 °C. The maximum operating pressure was determined with the injection valve first switched to the loading position (SI, **Fig. S2 C**). After each new flow rate was set, the value of pressure was recorded, while observing that the pressure was steady and no leakage occurred. The injection valve was then switched to the injecting position (SI, **Fig. S2 D**) for 20 minutes before being switched back to loading position. The injection valve was finally tested under 32MPa (4640 psi) for 24 hours, which is the maximum operating pressure of the Dionex ICS-5000 system used in this experiment. The detailed experimental setup is illustrated in SI, **Fig. S4 D**.

2. The schematic of the miniaturised LC system

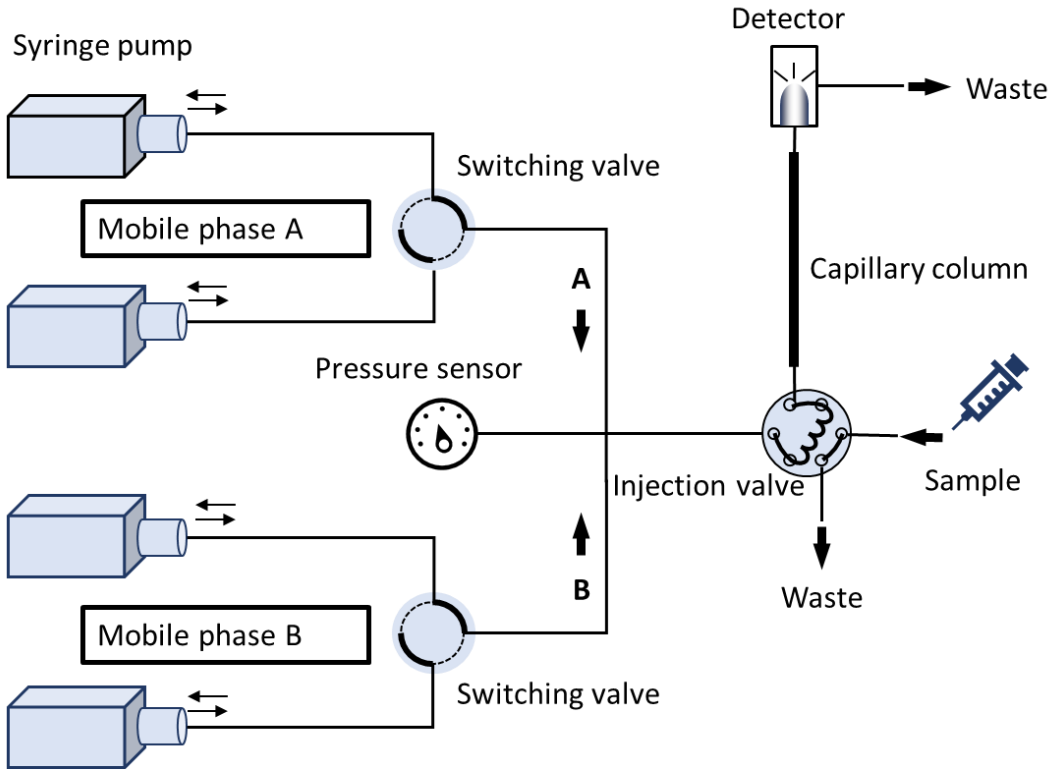

Fig. S1. The schematic of the miniaturised LC system.

3. Summary of specifications for conventional injection valve and the miniaturised injection valve.

Table S1. Summary of specifications for conventional injection valve and uProcess™ miniaturised injection valve.

Valve type	Internal volume (nL)	Maximum pressure (MPa, psi)	Switching time(ms)	Valve material	Size (cm^3)[a]	Weight (g)	Price (US$)[b]
Conventional injection valve	130 – 336 [1]	Up to 137.9 (20000) [2]	Ca. 150 [3,4]	Stainless steel	420.5	860	1500
uProcess™ injection valve	100 nL (manufacturer specification) 98 nL (measured)	34.5 (5000) (manufacturer specification) >32 (4800) (measured)[c]	250	Vespel	14.6	33	900

[a]Space effectively occupied by a box in which the instrument would fit as other works using this data [5].
[b]The costs are indicative price range for each instrument type judged across a variety of manufacturers active in each area.
[c]It is the highest investigated backpressure. The injection valve has the potential to operate under a pressure higher than 32 MPa (4641 psi).

4. The schematic of the miniaturised capillary injection valve (Rotor and rotor holder)

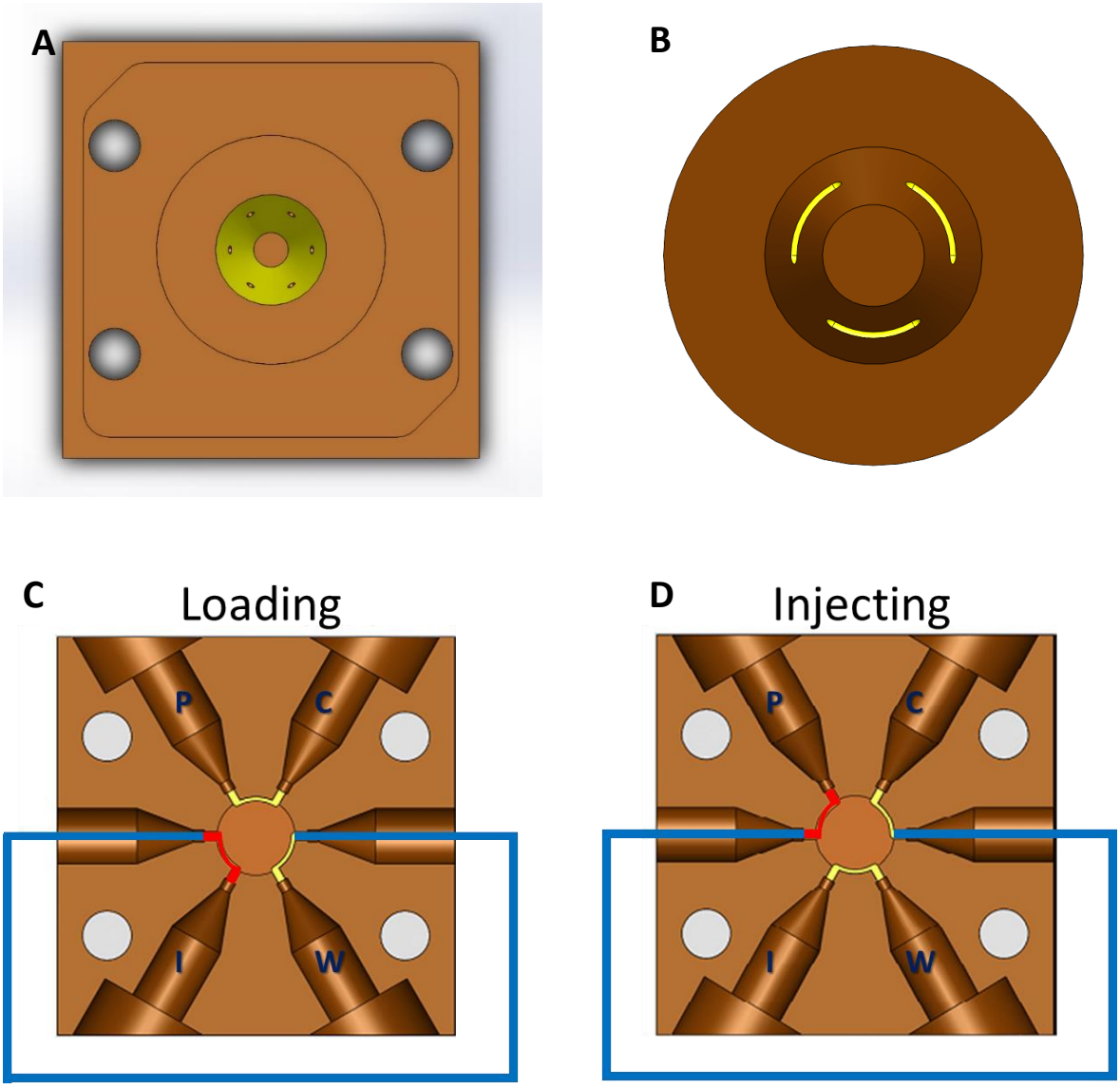

Fig. S2. The 2-D schematic and configuration of the miniaturised capillary injection valve (stem and stem holder). A. Stator. B. Conical-shaped rotor. C. Configuration of the injection valve at loading position. D. Configuration of the injection valve at injecting position. The red labelled areas in C and D indicate the internal volume (98 nL).

5. Schematic of the miniaturised capillary injection valve

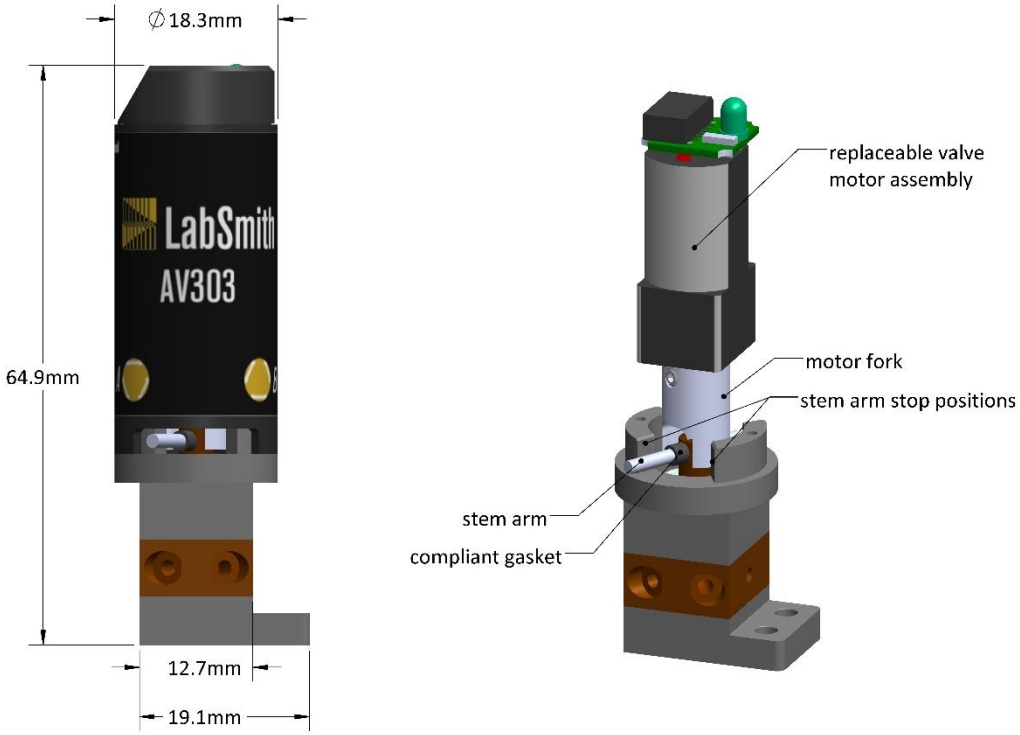

Fig. S3. The 2-D schematic and configuration of the miniaturised capillary injection valve.

6. The experimental setup for the study of injection performance under different backpressures

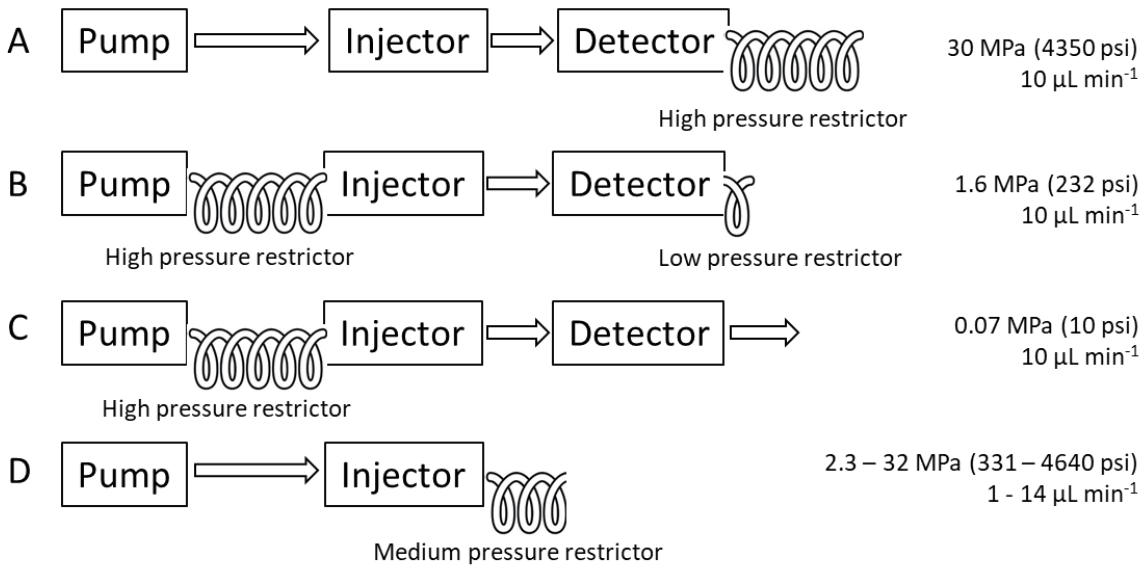

Fig. S4. The experimental setup for the study of injection performance under different backpressures (**A**, **B** and **C**) at the same flow rate (10 µL min^{-1}) and maximum pressure study (**D**) at different flow rates (1-14 µL min^{-1}). Three pressure restrictors were used: high pressure restrictor (10 µm x 44 mm), medium pressure restrictor (10 µm x 33 mm) and low pressure restrictor (25 µm x 90 mm). The solvent used was deionised water. Temperature: 25 °C. For **A**, **B** and **C**, the pump always operated against the same backpressure to provide a similar performance. The C4D on-column detector was connected at the shortest possible distance from the injection valve.

7. Internal volume

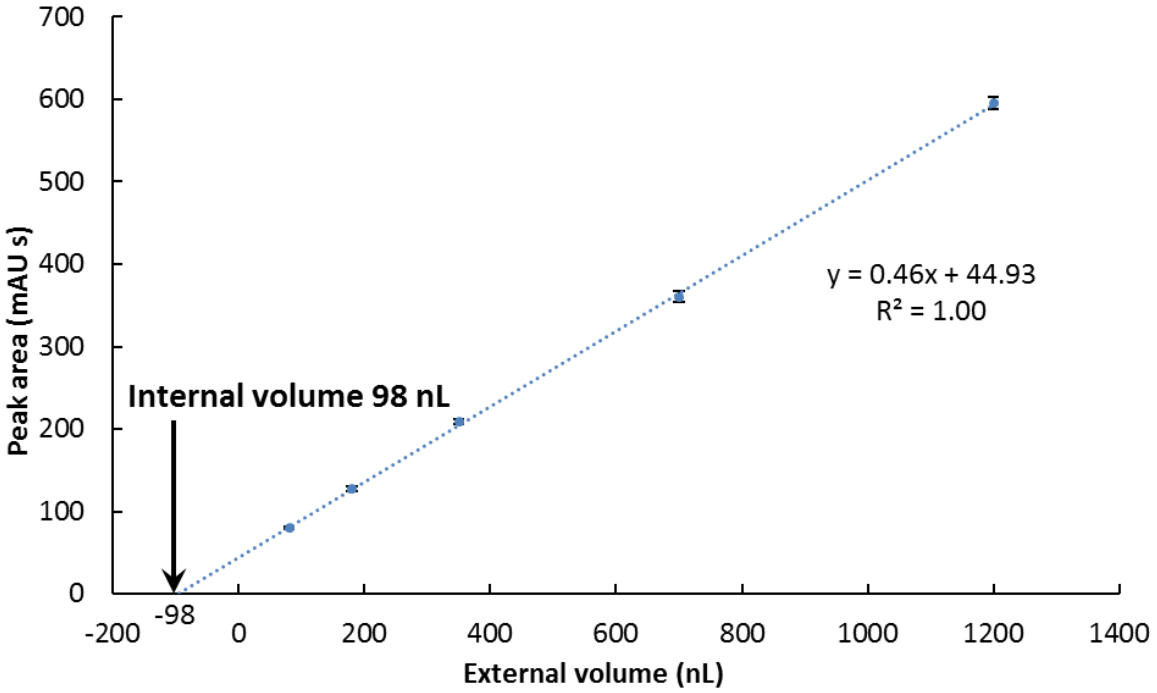

Fig. S5. The calibration curve of peak area (uracil, 1 mg L^{-1}) vs the volume of external sample loop. Flow rate 1 µL min^{-1}. The internal volume (98 nL) was determined by extrapolating the calibration curve to peak area at zero (calibration curve intercept across x-axis). For details of conditions see Experimental in SI.

8. Maximum operating pressure

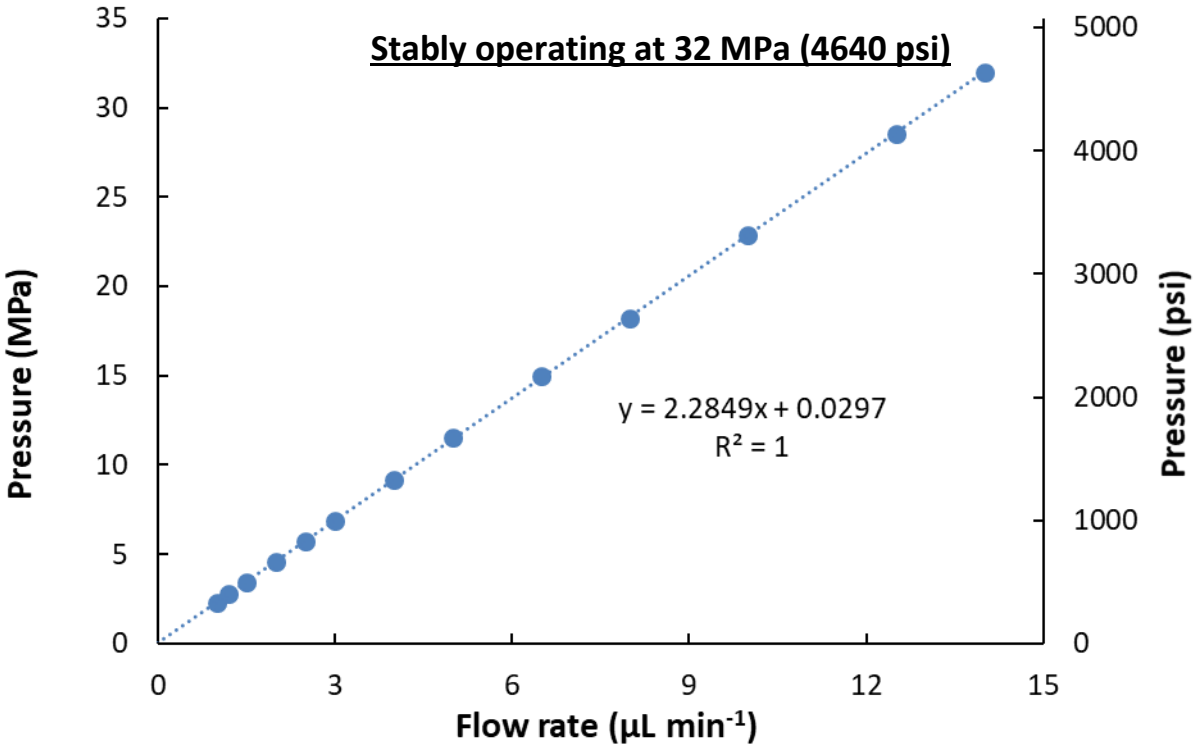

Fig. S6. The measurement of maximum pressure of the miniaturised injection valve. The injection valve can be stably operated at 32 MPa (4640 psi) without any leakage being observed. Details of conditions see Experimental.

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2.2 Performance of a miniaturised syringe-based solvent delivery system for portable capillary LC

2.2.1 Introduction

Solvent delivery system is one of the most crucial components for flow analysis instruments, such as LC and flow injection analysis (FIA), as it determines the fluidic performance and applicability of an instrument. The important parameters of a solvent delivery system include maximum pressure of operation, the precision and accuracy of the fluid flow and the flow rate scale. Modern reciprocating pumps for UHPLC have achieved maximum operating pressures of 140 MPa (20000 psi) while producing pulse-less flow at accurate flow rates[1-2]. Over the last decades, trends towards miniaturised and portable analytical instrumentation put notable attention onto miniaturised eluent delivery systems with increasing market interest. However, the bulky dimensions of benchtop reciprocating pumps make them impractical for miniaturised devices.

The difficulty of implementing reciprocating pumps in miniaturised devices redirects the interest onto syringe pumps. As syringe pumps have simple designs, they are relatively easy to miniaturise and implement on portable devices. Several studies in the development of miniaturised syringe pumps have been reported[3-4]. Furthermore, syringe pumps became the choice of some miniaturised and portable LC devices[5-10]. However, syringe pumps usually produce pulses that affect the flow rate due to the stepper motor action. The fluctuations of the flow rate will negatively impact especially on the detection baseline noise. To lower the short-term flow fluctuations, pulse dampers are often used[11].

An early study found that with the presence of an air bubble in the syringe, the flow rate fluctuation can be eliminated[11]. However, while this method provides a simple solution for low-pressure syringe pumps, is not practical for high pressure flow systems, as the air bubble will be gradually absorbed in the eluent liquid and so lose the damping function. For a fluidic flow system operated under pressure used in our previous study on miniaturised capillary LC, we showed that the flow rate fluctuations decreased dramatically by over an order of magnitude with relatively small backpressure of only 0.35 MPa (43.5 psi), applied through a backpressure restrictor, while a chromatographic column achieved the same effect[9].

In this study, the impact of back pressure on syringe pump flow rate fluctuation was investigated. The syringe pump studied here was a miniaturised microsyringe pump for microfluidic platforms, which we further developed to work within a medium pressure LC system. It has a highly compact design making it currently one of the smallest and lightest commercially available high pressure microsyringe pumps. This study focused on the cause of the flow rate fluctuation observed at very low backpressure and the effect of back pressure damping. Finally, methods of reducing the syringe pump fluctuation are presented and discussed.

2.2.2 Experimental

2.2.2.1 Chemicals and reagents

Deionised water (Millipore, Bedford, MA, USA) were used for testing the pump performance.

2.2.2.2 Instrumentation

12 programmable microsyringe pumps with one 5 µL syringe (SPS01, LabSmith, Livermore CA, USA) were used in this study. The pumps were plugged in LabSmith uProcess™ microfluidic system ("Microfluidic Fluid Control & Connectors"[12], LabSmith, Livermore CA, USA) and tested one by one. This microfluidic platform also consists an AV201 automated 3-port microfluidic switching valves (LabSmith, Livermore CA, USA). More detailed descriptions can be found from the manufacturer specifications[13]. For monitoring the flow rate, a nano-flowmeter (Upchurch) was used. The restrictors for testing the injection valve operation at different backpressures and maximum operating pressure were made of polyimide coated fused silica capillary (variable length; 25 µm i.d., Polymicro Tech; Phoenix, AZ, USA).

Software from uProcessTM (LabSmith) was used for driving the LabSmith components. The software from Upchurch “USI Nano Flow” was used to view and monitor the flow rate on the computer. All the data were recorded at 20 Hz.

2.2.2.3 Methods and procedures

For the air bubble damping, the damping air bubble was firstly slowly drawn into the syringe of the syringe pump on the Labsmith microfluidic system. This air bubble was sitting just next to the Teflon piston tip of the syringe of the syringe pump on the Labsmith microfluidic system, where the air bubble remained throughout the experiment[11].

2.2.3 Results and Discussions

2.2.3.1 Flow rate pulsation investigation

The flow rate pulsations of syringe pumps is thought to be caused primarily by the steps of the stepping motor, while the different pulsation profiles between pumps was reasoned to be due to the different combinations between piston tips and syringe bodies[5]. To develop a flow rate damping method, it is necessary to develop a thorough understanding of the flow rate pulsation mechanisms. Therefore, flow rate profiles of 12 syringe pumps were recorded (**Fig. 2.1**). Different to previous studies, in our study, 12 different syringe pump motor units (including the attached microgears, further the mechanical driving unit will be here termed “motor units”) were assembled individually in each experiment with only one glass syringe and piston set (the syringe with the piston was always the same). Therefore, the differences between the observed flow rate profiles can be only due to the differences in the performance of the syringe pump motor.

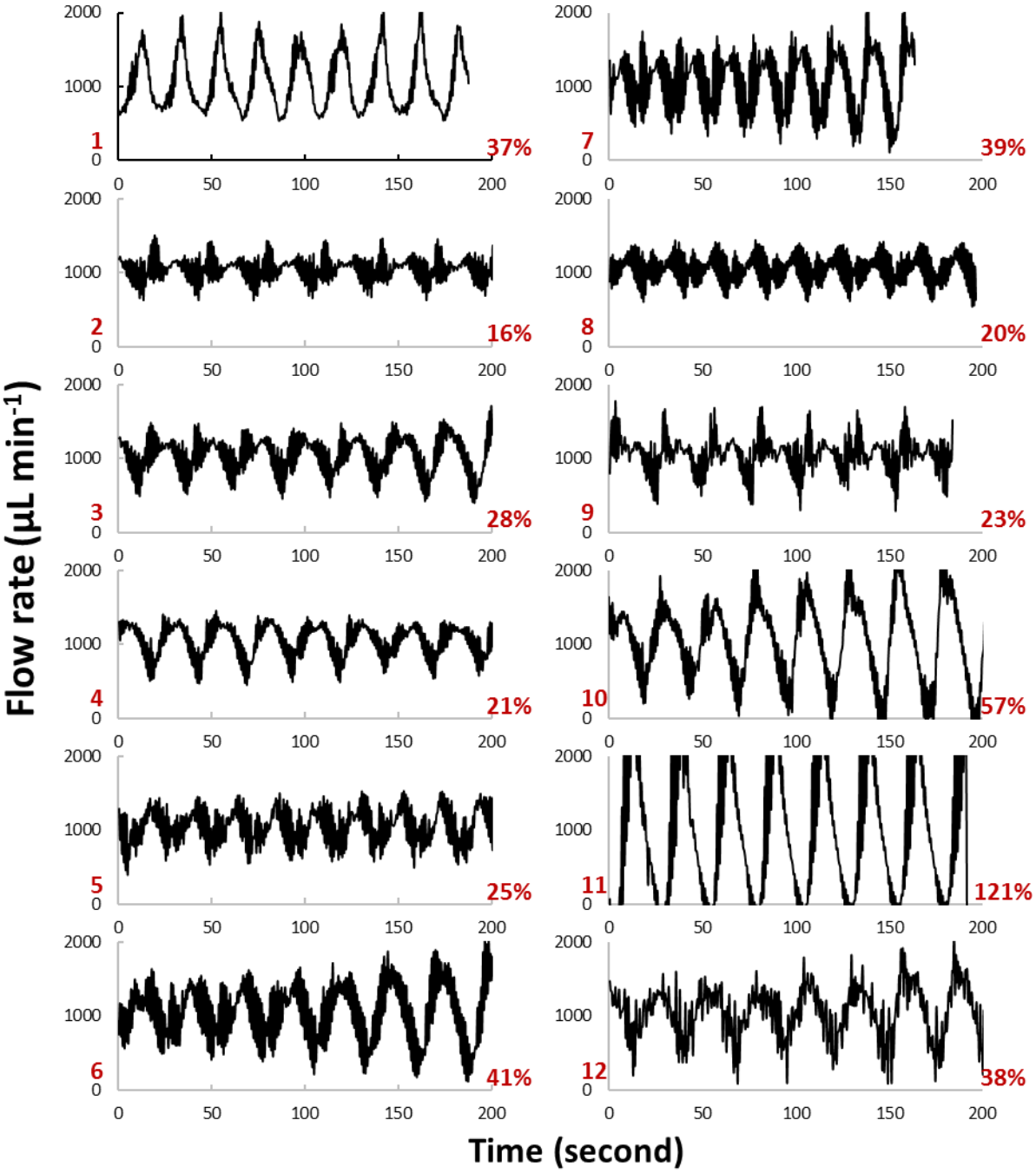

Fig. 2.1 Flow rate pulsation profiles of 12 motor units assembled a same 5 µL glass syringe. The flow rate pulsations (as %RSD) varied from 16% to 121% for different motor units. Set flow rate: 1 µL min^{-1}, Pressure < 0.01 MPa. Details of conditions see **Experimental**.

The result showed that even with the same glass syringe and piston set, different motor units gave different flow rate profiles. The flow rate pulsations (as %RSD) varied from 16% to 121% for 12 motor units. This suggested that the motor units contributed to the flow rate pulsation significantly, and different motor units had their unique "fingerprint". This was believed due to the movement of piston driven by the mechanical motor force. Therefore, to damp the syringe pump flow, it is necessary to resolve the uneven mechanical movement issue.

2.2.3.2 The pressure damping investigation

The bubble damping system developed in previous study[3] showed good smoothing performance for low pressure system (**Fig. 2.2**). However, as stated in the **Introduction**, the air bubble damping method is not practical for high pressure system. Therefore, in this section, the pressure damping performance was investigated.

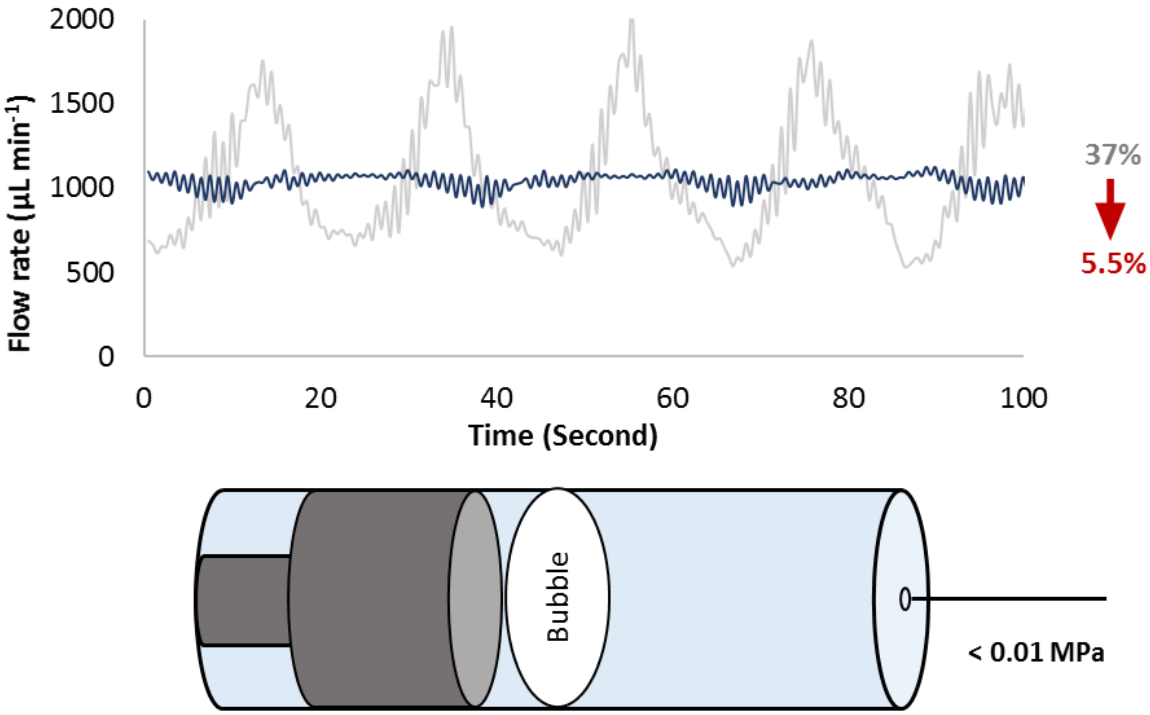

Fig. 2.2 The performance of air bubble damping system (gear number 1 in **Fig. 2.1**). The damping system reduced the flow rate pulsations (as %RSD) effectively from 37% (grey line) to 5.5% (blue line). Bubble size 1 µL. Set flow rate: 1 µL min^{-1}, Pressure < 0.01 MPa. For details of conditions see Experimental.

Three motor units with different flow rate pulsation levels were selected for pressure damping study (motor unit number 1, 2 and 4 in **Fig. 2.1** using a same glass syringe). It was found that, under 1.5 MPa pressure, the flow pulsations reduced significantly for all three motor units. In addition, even though three motor units produced the flow pulsation at different levels, after the pressure damping, they all perform similarly, which only produced 4.6%-2.5% comparing to 37%-16% without pressure. The reason for the pressure damping mechanism was thought to be that the backpressure provided a force against the stepper motor movement, which smoothened the forward movement of the piston. To compare the performance of pressure damping with the bubble damping, the bubble damping study was also conducted. With 1 µL air bubble presented in the syringe, for the same motor unit (motor unit number 1), the flow rate

pulsations (as %RSD) was reduced from 37% to 5.5% (**Fig. 2.3**), while for the pressure damping, the flow pulsation for the same motor unit was further reduced to 4.4%.

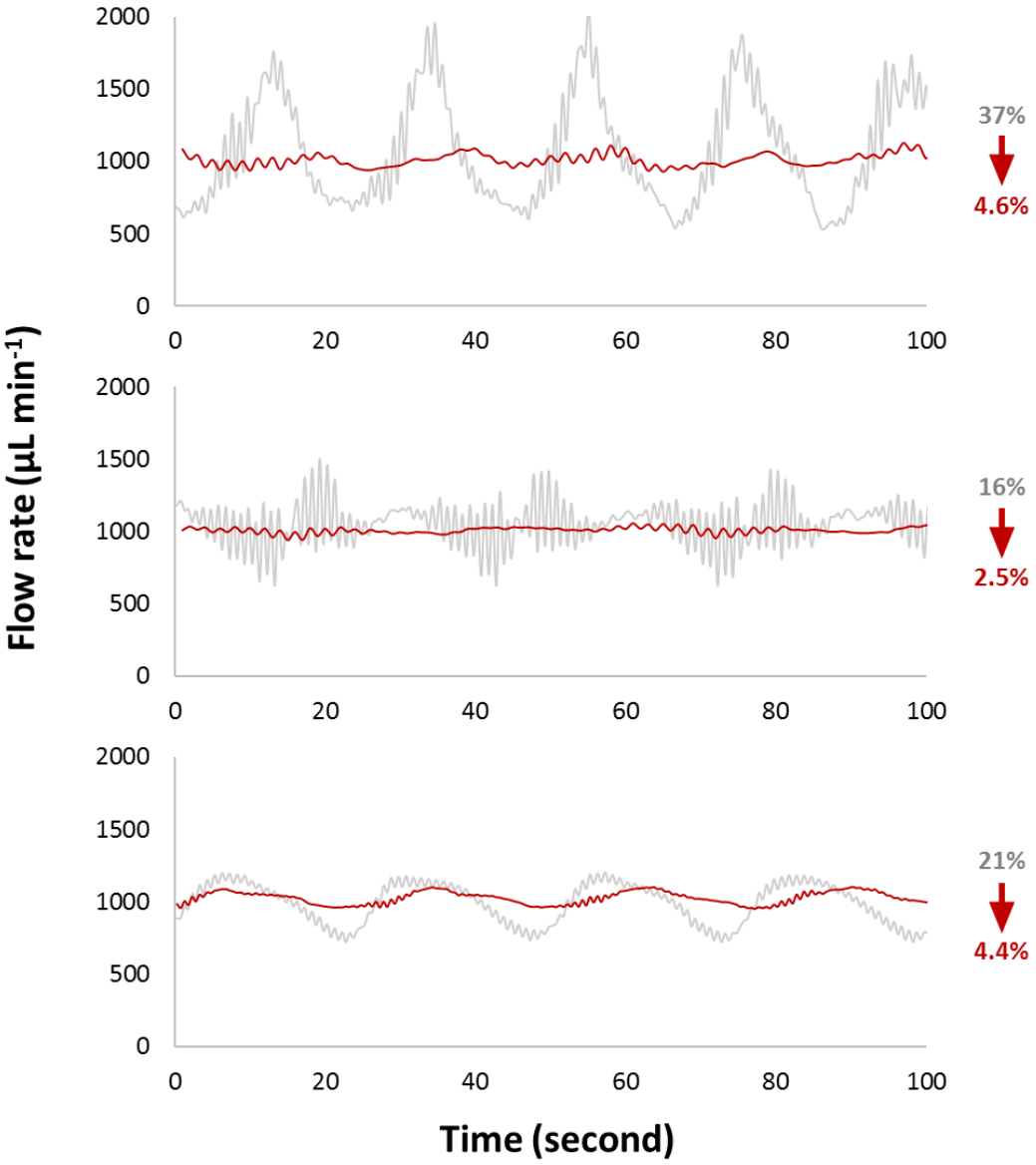

Fig. 2.3 The performance of pressure damping system (motor unit number 1, 2 and 4 in **Fig. 2.1**). Before pressure damping grey line, after pressure damping red line. The damping system reduced the flow rate pulsations (as %RSD) effectively. Set flow rate: 1 µL min^{-1}, Pressure 1.5 MPa. Details of conditions see Experimental.

In the chromatographic system, the column itself can be acted as the pressure restrictor, therefore, here the syringe pumps proved to be suitable pump option for the LC eluent delivery system. The flow pulsation was found highly due to the motor mechanical movement pulsation and can be smoothened by force. For the low pressure fluidic device (e.g. FIA), a resist component can be applied on the syringe pump motor unit to provide a force to smooth the motor movement.

2.2.4. Conclusions

This study into pulsations of miniaturised microsyringe pumps for microfluidic platforms investigated the cause of the flow rate fluctuations observed at very low backpressure, and the effect of back pressure damping is useful when working under significant backpressure conditions such as in LC. We found that the motor units contributed to the flow rate pulsation significantly, and different motor units had their unique “fingerprint”. Importantly, pressure damping at pressures as low as 1.5 MPa reduced the flow fluctuation by over an order of magnitude. Therefore, the syringe pumps can be suitable for LC eluent delivery systems, as the column itself will act as a pressure restrictor.

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Chapter 3. Comparison of cation-exchange capillary columns used for ion chromatographic separation of biogenic amines

This chapter is to investigate the options of suitable narrow-bore or capillary chromatographic columns. It was necessary to find columns with small dimensions, high separation efficiency, minimum sample volume needs and very low solvent consumption. Additionally, the column needed to be capable of operation under medium backpressure.

*All of this research contained in this section has been published as Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., Comparison of cation-exchange capillary columns used for ion chromatographic separation of biogenic amines. *Journal of Chromatography A* **2018,** *1571*, 193-200.

Chapter 4. Deep UV-LED photometric detection for miniaturised LC

4.1 High power deep UV-LEDs for analytical optical instrumentation

*All of this research contained in this section has been published as Li, Y.; Dvořák, M.; Nesterenko, P. N.; Nuchtavorn, N.; Macka, M., High power deep UV-LEDs for analytical optical instrumentation. *Sensors and Actuators B: Chemical* **2018,** *255,* 1238-1243.

Supplementary Information (SI)

High power deep UV-LEDs for analytical optical instrumentation

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1. Photometric on-capillary detection study: sensitivity vs. absorbance curve and resulting effective pathlength and stray light

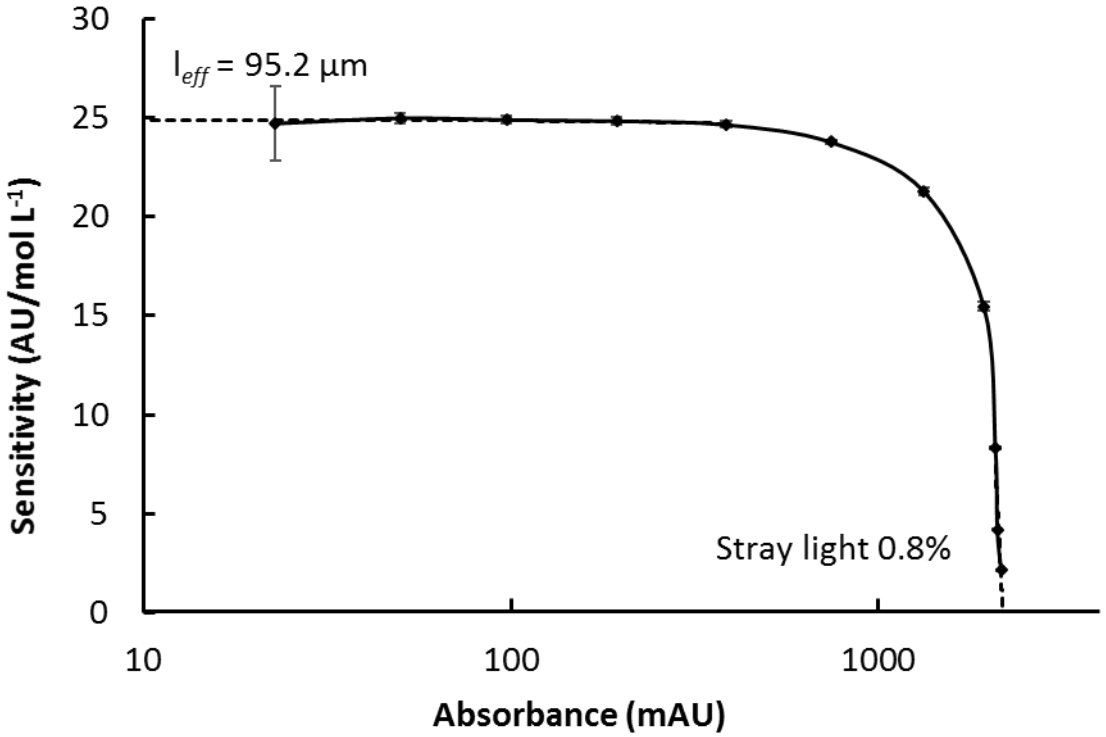

Fig. S1. Detection linearity as sensitivity vs. absorbance graph. Conditions: Capillary inner diameter (100 µm), test analyte chromate, effective pathlength (l_{eff} = 95.2 µm) calculated for sensitivity extrapolated to A=0 equal to 25 AU/mol L^{-1}, and chromate molar absorptivity coefficient =2624 L mol^{-1} cm^{-1} as measured. The stray light 0.8% from the extrapolated high-end of the curve at A = 2.178. An upper limit of detection (LOD), determined as the absorbance corresponding to 95% sensitivity, was calculated as 745 mAU.

2. The analyte spectra overlayed with the emission spectrum

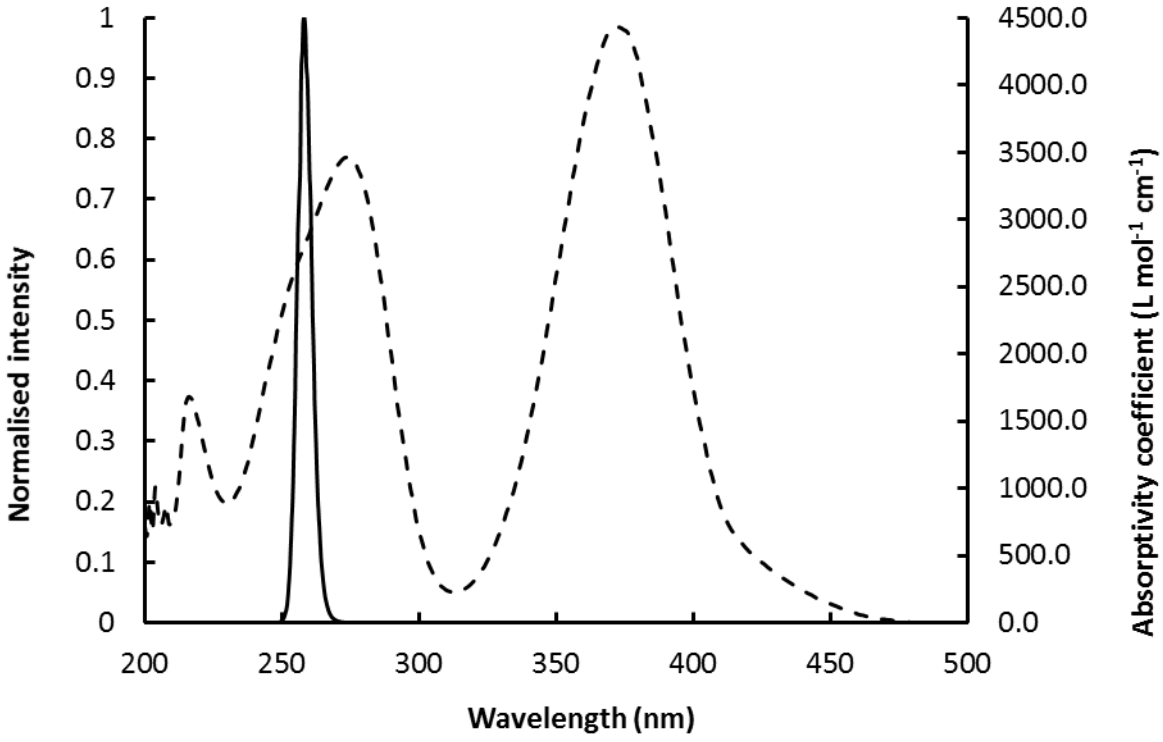

Fig. S2. Absorbance spectrum of chromate absorbing strongly at 255 nm overlayed with the 255 nm deep UV-LED emission spectrum.

3. Demonstration of detection performance

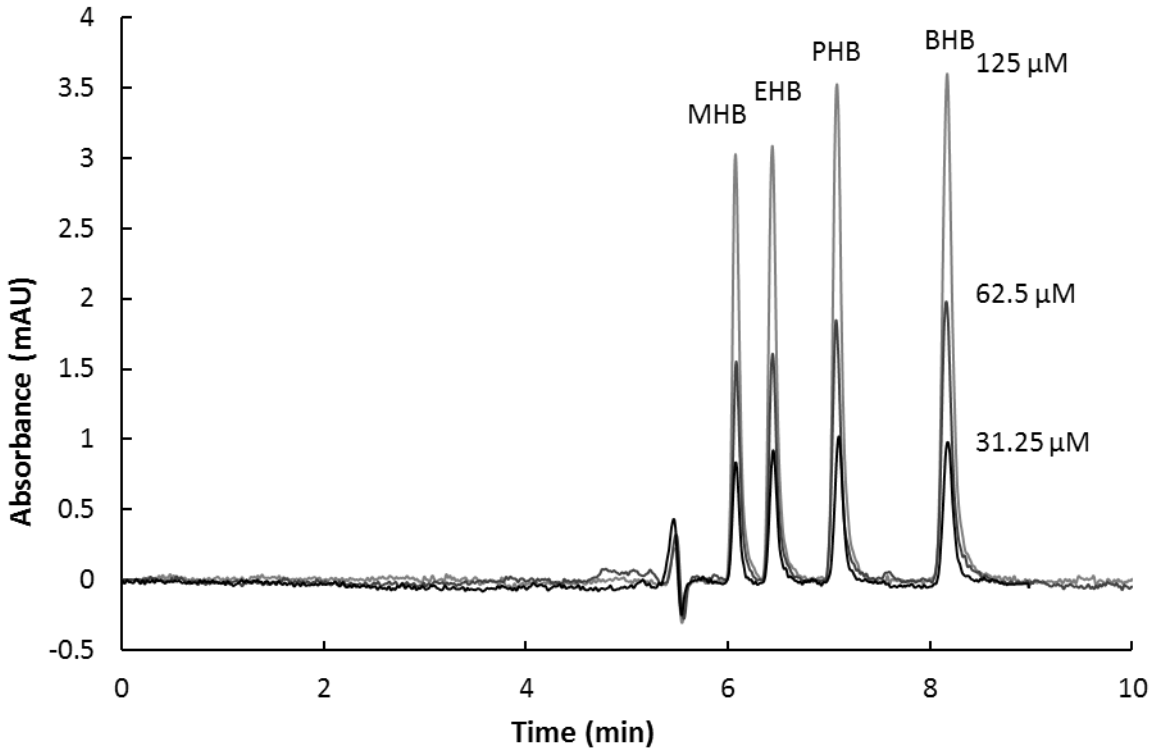

Fig. S3. Isocratic separation of parabens of different concentrations. Conditions: Concentration of all analytes in three separations was 31.25 µM, 62.5 µM or 125 µM. methyl 4-hydroxybenzoate (MHP), ethyl 4-hydroxybenzoate (EHB), mM propyl 4-hydroxybenzoate (PHB), and butyl 4-hydroxybenzoate (BHB); eluent: 50 mM ammonium acetate - acetonitrile 50/50 (v/v); flow rate: 0.5 mL min^{-1}; column: 30 cm × 100 µm i.d.; injection volume: 4 nL (sample injection length as in the on-capillary detector: 500 µm); detection: 255 nm LED on-capillary photometric detector, inserted capillary i.d. 100 µm, optical window width 50 µm. Deep UV-LED forward current 100 mA.

4.2 Performance of a new 235 nm UV-LED based on-capillary photometric detector

*All of this research contained in this section has been published as Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Li, Y.; Nesterenko, P. N.; Paull, B.; Stanley, R.; Macka, M., Performance of a new 235 nm UV-LED-based on-capillary photometric detector. *Analytical Chemistry* **2016,** *88* (24), 12116-12121.

Supplementary Information (SI)

Performance of a new 235 nm UV-LED based on-capillary photometric detector

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1. The temperature measurement using thermal imaging

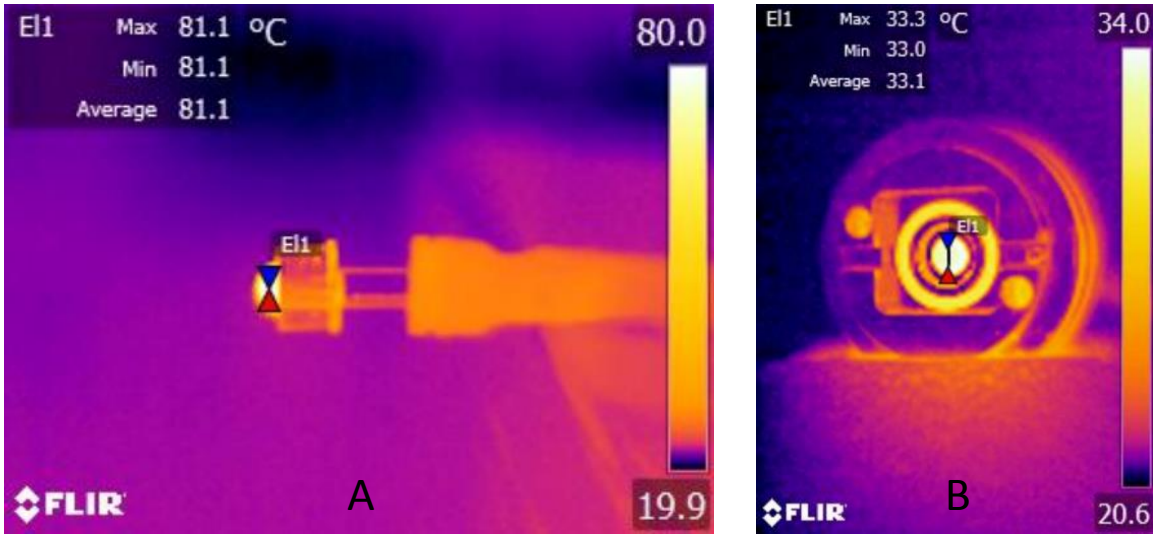

Fig. S1 Thermal image showing examples of temperature measurements of the 235 nm deep UV-LED. A: without heat sink at 7 minutes. B with heat sink at 4 hours. The temperature was measured at the outer surface of the integrated lens as labelled in the image.

2. The linear range for determination of iodide in simulated seawater

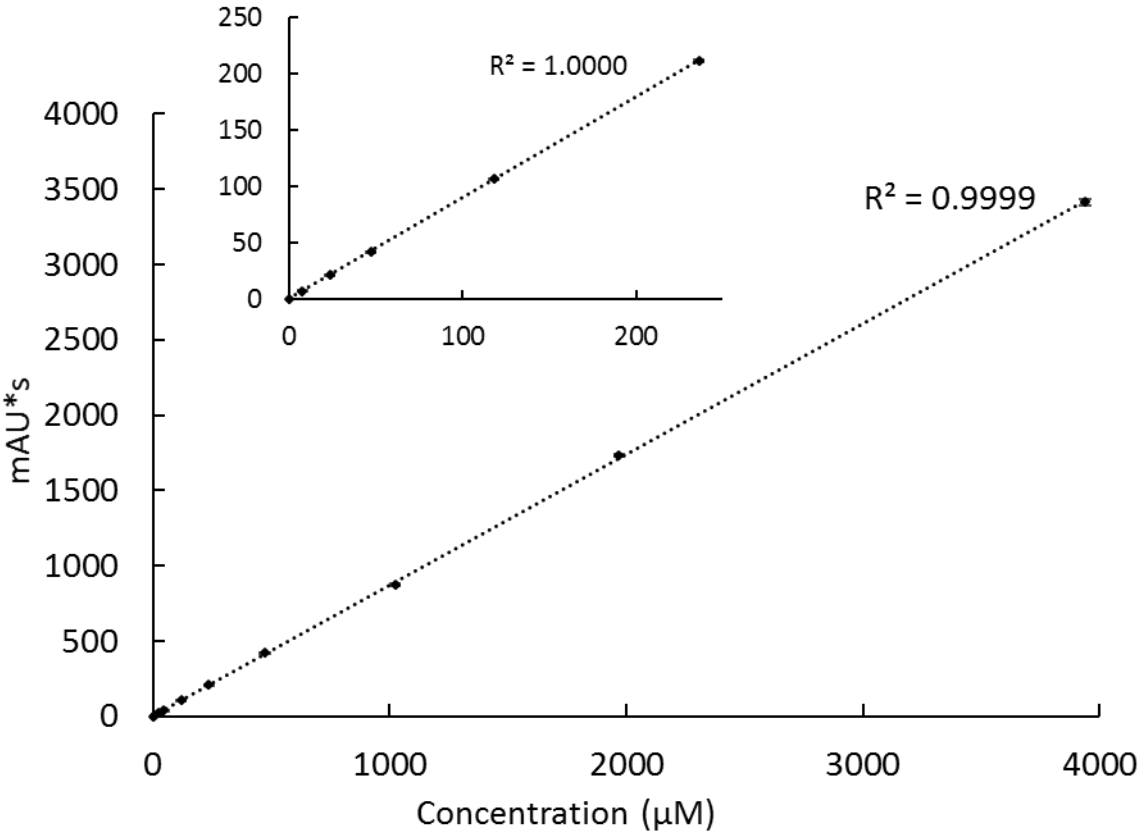

Fig. S2 Calibration curve of determination of iodide in simulated seawater using capillary IEC. 7.9 to 3937 μM L^{-1} linear range based on peak areas was achieved. Conditions are same as Fig. 4.

4.3 High sensitivity deep-UV LED-based z-cell photometric detector for capillary LC

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*All of this research contained in this section has been published as Li, Y.; Nesterenko, P. N.; Stanley, R.; Paull, B.; Macka, M., High sensitivity deep-UV LED-based z-cell photometric detector for capillary liquid chromatography. *Analytica Chimica Acta* **2018,** *1032*, 197-202.

Supplementary Information (SI)

High sensitivity deep-UV LED-based z-cell photometric detector for capillary liquid chromatography

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1. Schematic of the deep-UV LED based z-shaped flow cell detector with dimensions

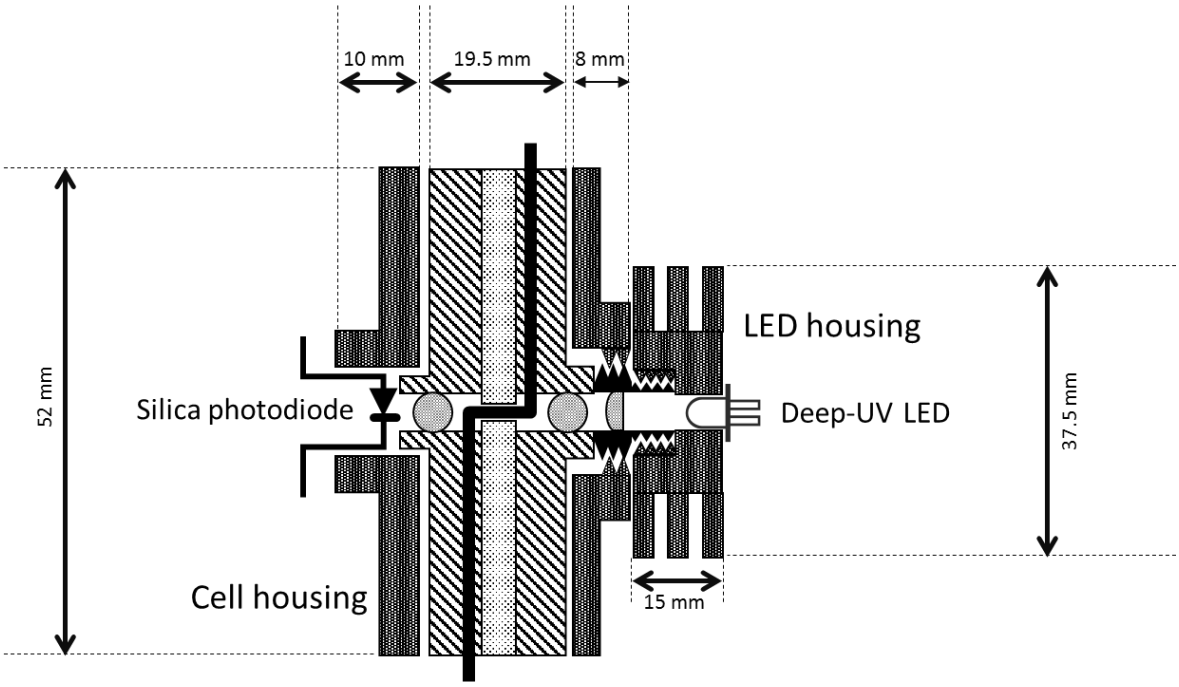
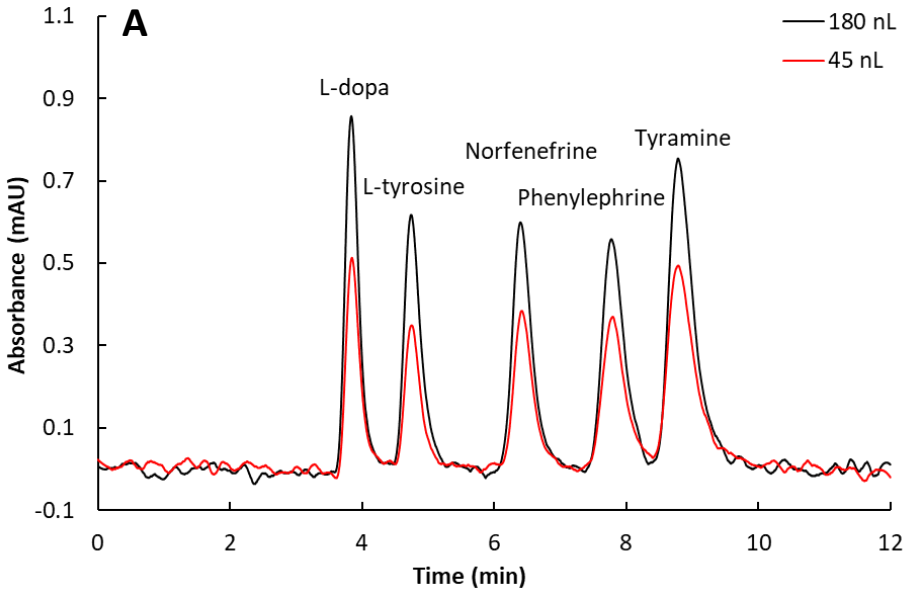


Fig. S1. Schematic of the deep-UV LED based z-shaped flow cell detector with dimensions.

2. Separation performance of the Z cell detector with 280 nm LED



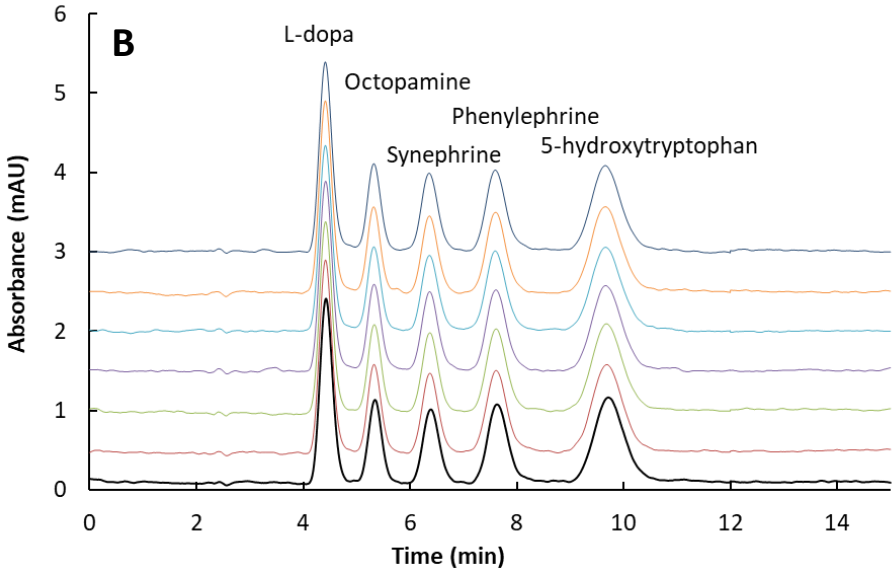


Fig. S2. The separation of biogenetic amines. LED wavelength: 280 nm. Other conditions were the same as **Fig. 4**.

Chapter 5. Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components

*All of this research contained in this section has been published as Li, Y.; Dvořák, M.; Nesterenko, P. N.; Stanley, R.; Nuchtavorn, N.; Krčmová, L. K.; Aufartová, J.; Macka, M., Miniaturised medium pressure capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components. *Analytica Chimica Acta* **2015,** *896*, 166-176.

Electronic Supplementary Information (ESI)

Portable medium pressure portable capillary liquid chromatography system with flexible open platform design using off-the-shelf microfluidic components

Yan Li, Miloš Dvořák, Pavel N. Nesterenko, Roger Stanley, Nantana Nuchtavorn, Lenka Kujovská Krčmová, Jana Aufartová, Mirek Macka

1. LabSmith AV series automated valves

Tab.S1 LabSmith AV series automated valves specifications [1].

Valve	Through hole diameter	Swept volume	Valve volume
AV201-C360	0.01” [250 µm]	130 nL	170 nL
AV201-T132	0.01” [250 µm]	130 nL	170 nL
AV201-T116	0.02” [510 µm]	520 nL	1.1 µL
AV202-C360*	0.01” [250 µm]	90 nL	130 nL

*The valve used in this work

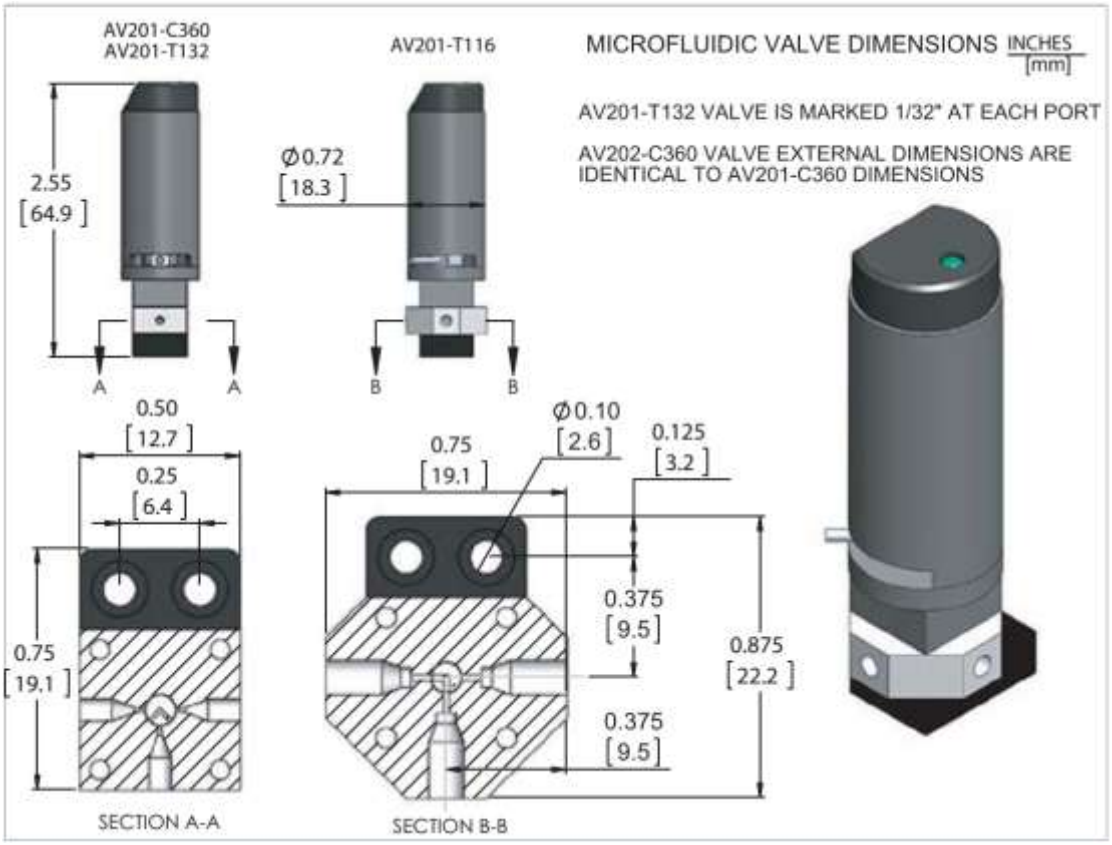


Fig. S1 LabSmith AV series automated valve technical specifications [1].

2. LabSmith SPS01 Syringe Pumps

Tab.S2.1 LabSmith SPS01 Syringe Pumps performance with different volumes of syringes [1].

Maximum volume (µL)	Minimum flow rate (µL min^{-1})	Maximum flow rate (µL min^{-1})	Step size (nL)	Maximum pressure (MPa)
5*	130 nL	170 nL	8	> 3.4
10	130 nL	170 nL	17	> 3.4
20*	520 nL	1.1 µL	33	> 3.4
50	90 nL	130 nL	83	2.1
100	1.0	5600	170	2.1
Volume accuracy		1% (infuse direction)		
Flow rate accuracy		1% (infuse direction)		
Service temperature range		10 – 80 °C		

*The syringes used in this work

Tab.S2.2 Physicals of LabSmith SPS01 Syringe Pumps [1].

	Physical
Dimensions	100 * 25 *20 mm (L * W * H)
Housing material	Delrin®
Syringe material	Glass with PEEK™ tip
Plunger material	Stainless steel with Teflon® tip
Wet volumes	5, 10, 20, 50 and 100 µL (interchangeable, all volumes supported with the same housing)
Stroke length	12 mm
Tip interface	Directly connects to 360 µm o.d. capillary via CapTite C360-100 one piece fitting; or 1/16” o.d. tubing for selected
Cleaning	Wetted parts can be chemically sterilized or autoclaved

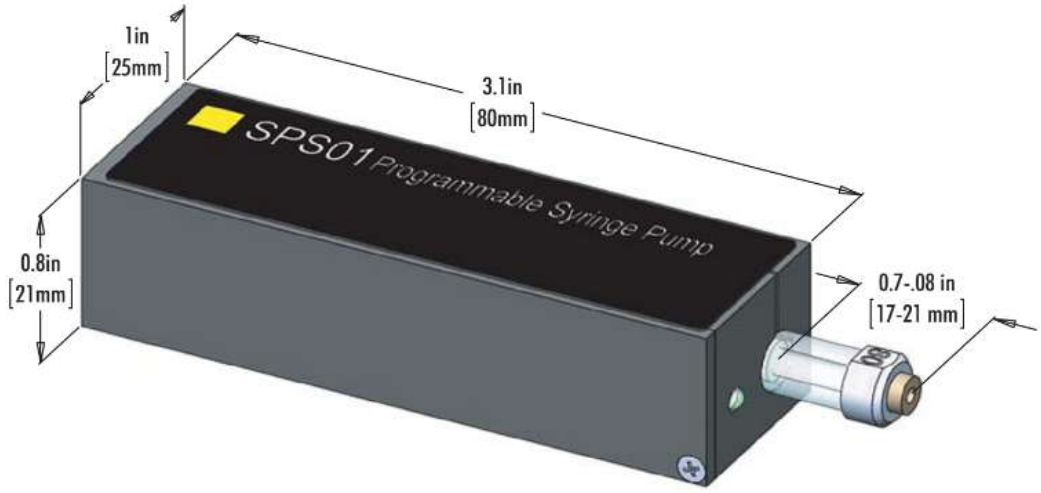


Fig. S2 LabSmith SPS01 Syringe Pump dimensions [1].

3. The calculation of the theoretical maximum pressure of the syringe pumps

When the glass syringe changed a known volume by moving the piston, the movement of the piston was visualised and measured by a microscope. Given that the volume was known, therefore, the cross-section area of the tip can be calculated. The measurement and calculation for 5 µL glass syringe are illustrated blow as an example. 20 µL and 100 µL syringes were used the same method.

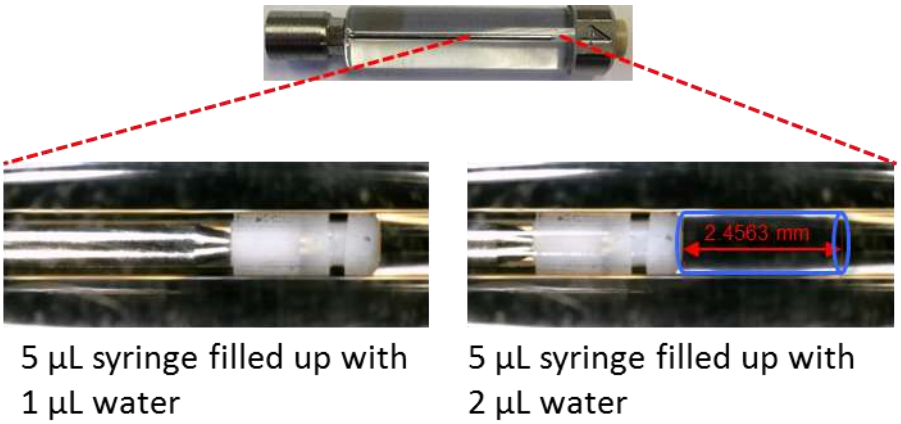


Fig. S3.1 Illustration of the calculation of the theoretical maximum pressure of the syringe pumps.

$S = V/L \Rightarrow S = 1\ \mu L/2.46mm = 0.41\ mm^2$

$S\ (mm^2)$ is the cross-section area, V (µL) is the volume, L (mm) is the distance of the movement

Tab. S3 the cross-section area of the piston tip and the theoretical maximum pressure

Volume of syringe (µL)	Maximum Force (N)	Cross-section area of the piston tip (mm^2)	Maximum pressure (MPa)
5		0.41	17
20	7	1.70	4
100		8.30	0.8

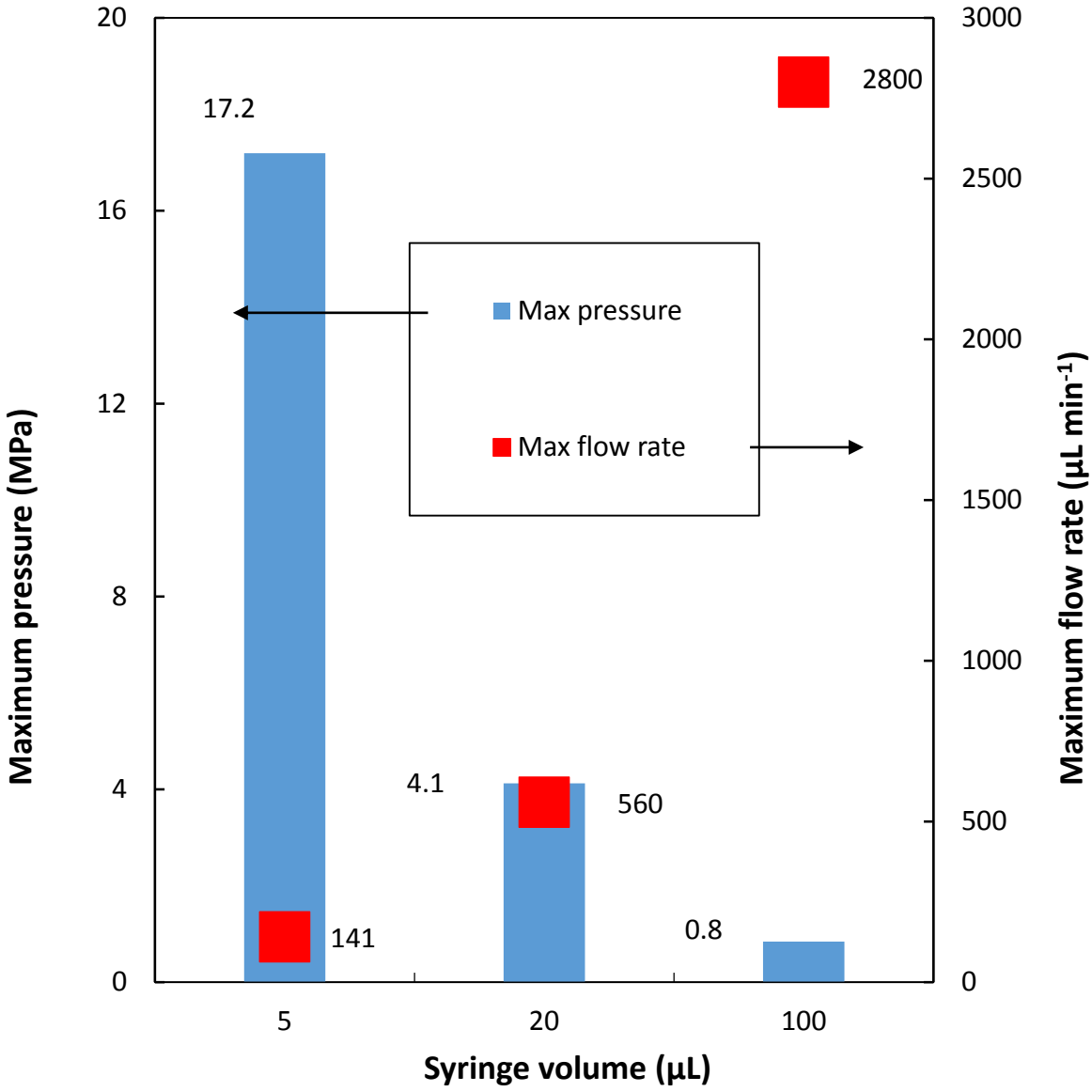


Fig. S3.2 The theoretical maximum pressure and the maximum flow rate for the three different syringes.

4. The measurement of maximum pressure for 20 µL syringe.

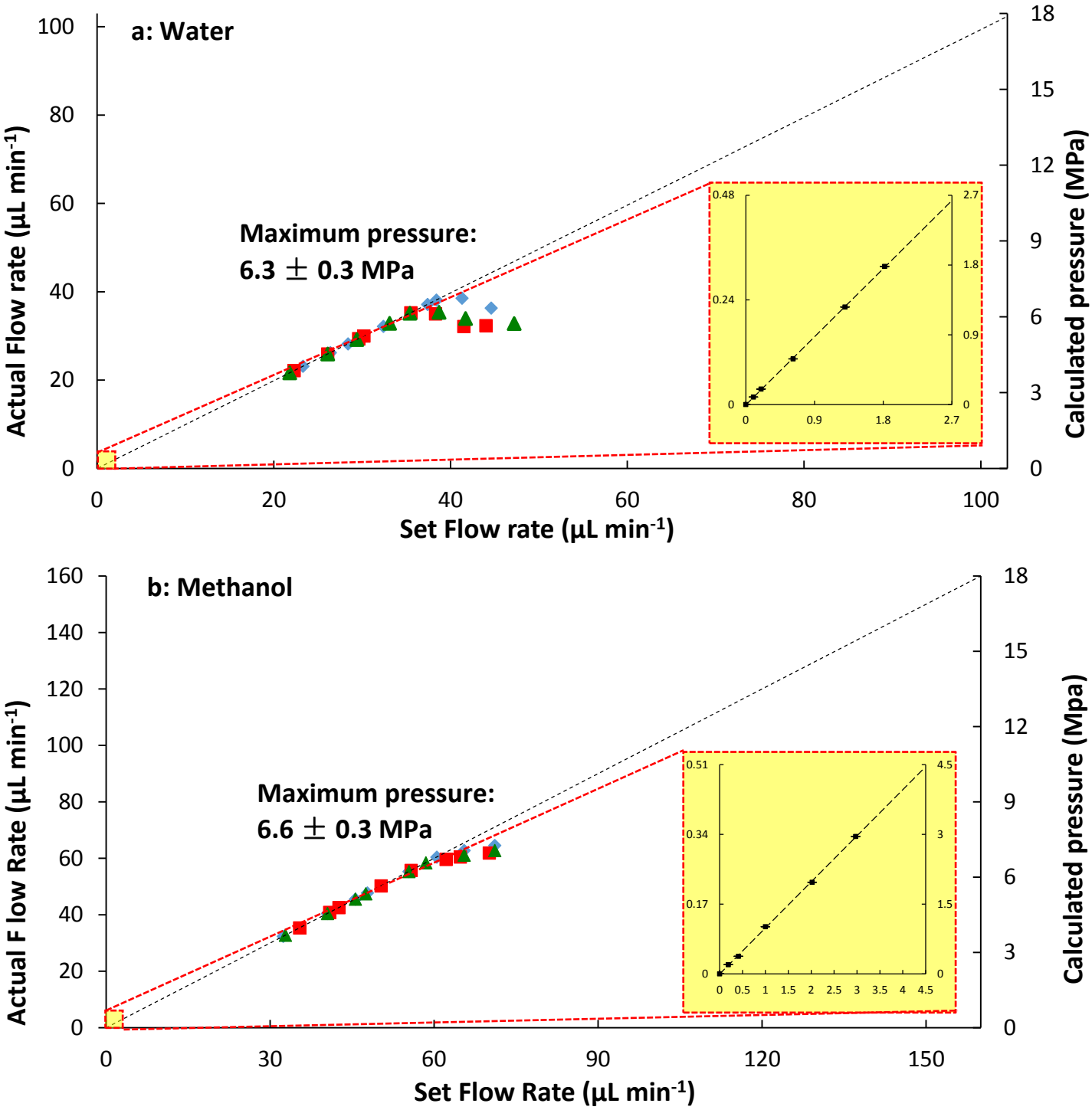


Fig. S4 Graph of pressure versus flow rate for 20 µL syringe micropumps. Pressure above 0.4 MPa were determined by extrapolating the calibration plot (insert) of measured backpressure by pressure sensor versus experimentally determined flow rates. For other conditions see Experimental.

5. Pumping system eluent composition performance (using 20 µL syringe)

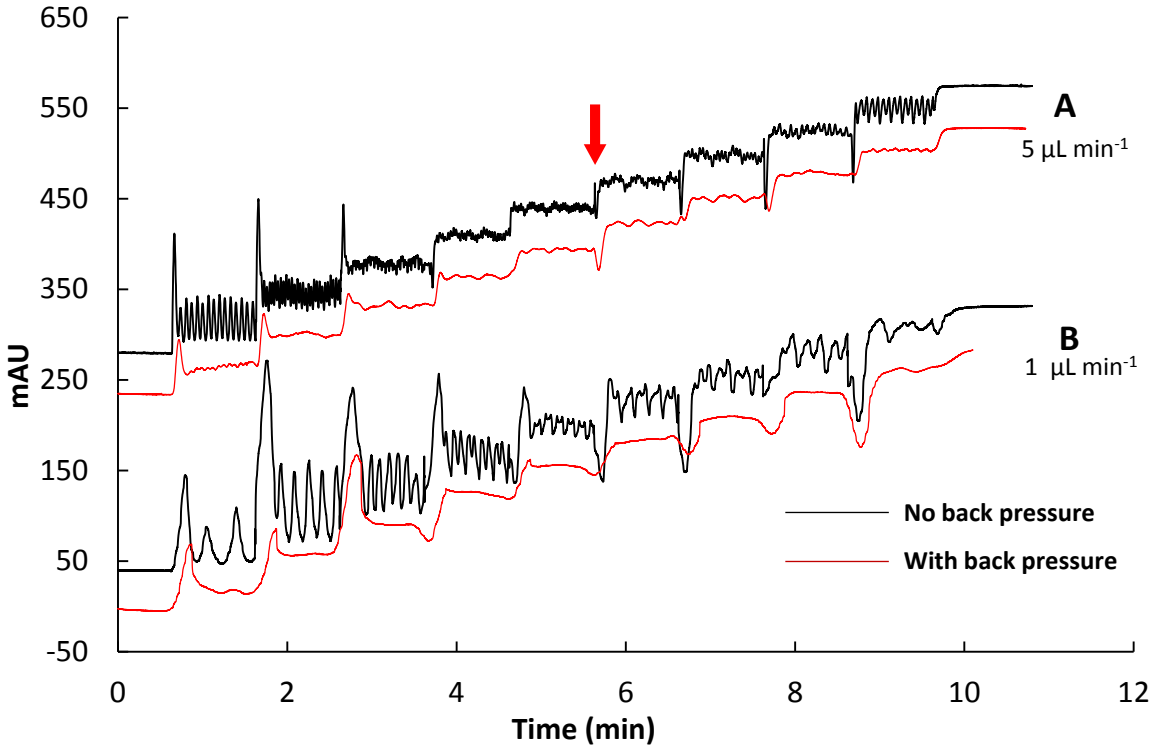


Fig. **S**5: Performance of syringe pumps using 20 µL syringes at different flow rates judged by visualised step gradient in steps of 10% from 0 to 100%B using an absorbing phase B; the arrows show the time when valves were switching. Conditions: Pump A1&A2: water, pump B1&B2: tartrazine 5 mM; LED detection: blue LED at 470 nm. For other conditions see Experimental.

6. Linearity and calibration plots from peak area (mAU) measurements

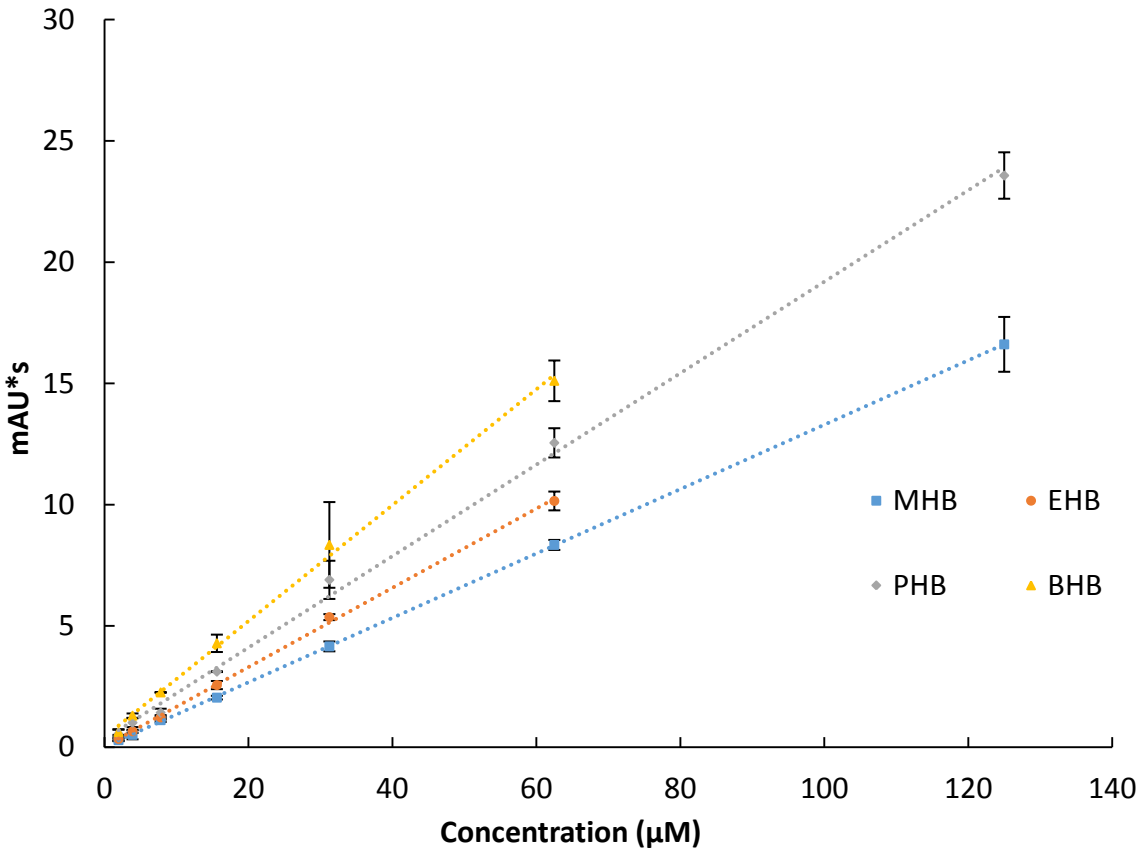


Fig. S6 Calibration plot (n = 3) for methyl 4-hydroxybenzoate (MHB), ethyl 4-hydroxybenzoate (EHB), propyl 4-hydroxybenzoate (PHB) and butyl 4-hydroxybenzoate (BHB). Eluent: 50 mM ammonium acetate - acetonitrile 8050/5020 (v/v); flow rate: 1 0.5 µL min^{-1}; column: 14 30 cm × 100 µm i.d.; injection volume: 4 nL; detection: 255 nm LED on-capillary photometric.

Tab. S6 Linearity Date from Peak Area (mAU) measurements

Analytes	Concentration range[a] (μM)	Regression equation	R^2	LOD[b] (nM)
MHB	1.95 - 125	y = 0.1328x + 0.016	0.999	174
EHB	1.95 – 62.5	y = 0.1637x + 0.0246	0.999	169
PHB	1.95 - 125	y = 0.1894x + 0.2705	0.998	141
BHB	1.95 – 62.5	y = 0.2414x + 0.3179	0.997	115

[a]The upper concentration was determined as the maximum concentration within the calibration standards still giving

[b]The Limit of Detection (LOD) was calculated using the signal to noise criteria S/N = 3

References

[1] http://labsmith.com/products/LabSmith%20uProcess%20Brochure.pdf (accessed 27/01 2015).

Chapter 6. Conclusions

The overall goal of a miniaturised or portable LC, as discussed in the **Introduction 1.5**, has been met. The overall performance of the miniaturised LC was found in most parameters to be comparable or superior to most other reported miniaturised LC systems, with clear potential for portable LC. The performance was understandably inferior to the standard commercial LC, mainly due to the miniaturised and low cost components. The miniaturised pumps are not able to deliver pressure higher than 2000 psi, which is the bottleneck of the development of the miniaturised LC. However, the performance is still satisfactory for the most of the on-site analysis applications. In addition, this low-cost system off-the-shelf approach may also have an excellent educational value especially given the modular, flexible and low-cost design philosophy. Further studies including the development of a miniaturised portable power supply system and appropriate data analysis system are needed to convert the current miniaturised system to be fully portable.

The future of portable LC might remain in relatively simple and rugged designs, while the application might divert into two directions: (i) the on in-field rapid analysis for environment monitoring, personnel health and security screening, (ii) as a component integrated in other comprehensive analytical machines, especially mass spectroscopy.

The advantages and principal achievements of this project comprise of:

6.1 Fluid delivery system

The array of four microsyringe pumps is used for the first time to mimic the design of classical piston type gradient eluent delivery system. The eluent delivery system has the advantages including (i) Syringe pumps operates in a continuous mode, (ii) the flow is defined / set, and (iii) the system is bio-compatible with no contact with metal parts.

The maximum operating pressure up to 11.6 MPa is sufficient for rapid analysis when using monolithic or short columns offering sufficiently low backpressures. The pressure damping mechanism based on presence of backpressure in the system significantly reduces the flow fluctuation of the syringe pumps, which make them capable of generating isocratic and gradient elution separations. An automated miniaturised splitless nano-flow pumping system that meets the requirements of minimum mobile phase consumption with solvent uses typically only 60 µL mobile phase per hour.

6.2 Sample introduction system

The evaluated injection valve is inherently superior in terms of its small size and low weight to other injection valves as its highly compact design makes it currently the smallest and lightest commercially available HPLC injection valve. However, it also shows a comparable level of performance to that of a standard HPLC nano-injection valve, with regard to its internal volume, carry-over, performance under different backpressures and maximum operating pressure.

6.3 Columns

The capillary column study allows a judicious choice of separation conditions for the characterised stationary phases and biogenic amines and amino acids in particular, but also in a more general way taking into account the properties of the columns and the solutes to be separated.

6.4 Deep UV-LED detection

The evaluated new generation deep UV-LEDs based on AlN substrate have ca. two orders of magnitude stronger intensity of optical power compared to the old UV-LEDs based on sapphire substrates. Due to the low parasitic-to-deep-UV (P/DUV) emission ratio, the new generation deep UV-LEDs can be employed in photometric detection without additional optical filters.

Our study also shows a first presented deep UV-LED emitting at a wavelength under 250 nm was capable of satisfactory performance as a light source for photometric detection in chemical analysis, and illustrates the potential that deep UV-LEDs obviously have as promising light sources for photometric detection in robust, miniaturised, low-cost analytical devices. The performance of the 235 nm deep UV-LED was found to be overall satisfactory, with particular regard to its emission spectra free of parasitic emissions, optical output, and low level of stray light and high upper limit of detector linearity when applied within an on-capillary detector. The higher current (100 mA) compared to older generations of UV LEDs or LEDs at higher wavelength necessitates use of cooling to prevent overheating of the LED resulting in loss of light.

The performance of the Z-typed flow cell detector was found to be overall satisfactory and promising for enhanced sensitivity detection, with particular regard to its effective pathlength, low level of stray light, baseline noise and high upper limit of detector linearity. The separation performance showed the detector had excellent reproducibility and sensitivity. The demonstration of the detector on a miniaturised capillary LC device showed its application for miniaturised analytical devices.

6.5 Miniaturised LC Platform

A miniaturised open platform of flexible modular design, using primarily low cost off-the-shelf components that are affordable and accessible to other researchers has been developed. Fluidic design flexibility was demonstrated as the modular design significantly widens the availability of such portable systems for other applications, with post-column reaction derivatisation or in-line sample treatment under investigation. On-site analysis potential is clearly given as the miniaturised LC analysis system can provide a capability to operate on site or in field analyses.